UNIVERSITY OF BRISTOL

THE ANNUAL REPORT

OF THE

Agricultural and Horticultural Research Station

(THE NATIONAL FRUIT AND CIDER INSTITUTE)

LONG ASHTON, BRISTOL
1951

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*Agricultural Research Council Plant Nutrition Scheme.

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*Agricultural Research Council Plant Nutrition Scheme.

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INTRODUCTION.

By T. WALLACE, Director.

During 1951 continued progress has been made in the various investigations comprising the research programme of the Institute.

The policy of close liaison with the local officers of the National Agricultural Advisory Service in their advisory work has been continued by personal contacts and by the participation of N.A.A.S. officers in scientific meetings held at the Station and organised by the Institute staff.

An annual refresher course for N.A.A.S. horticulture officers was again held at the Station on July 17th and 18th, when series of lectures were given by members of the Institute staff. The N.A.A.S. advisory chemists also visited the Station on July 11th to inspect the work in progress on plant nutrition, and officers of the general agriculture section of the service on September 25th to acquaint themselves with the general programme of work. For the second successive year a special pruning course for N.A.A.S. officers was given by Mr. F. A. Roach, Provincial Fruit Specialist for the South-Western Province of the N.A.A.S. and Mr. Hobbis, Station Plantations Officer.

Close relationships between the Institute and the N.A.A.S. Horticulture Experimental Stations concerned with fruit investigations have been maintained. The Director has acted as chairman of the Fruit Working Party set up to co-ordinate fruit experiments on these stations, and has also served on the Advisory Committees of the local stations. Good progress has already been made in the establishment of field experiments at the Luddington Station, near Stratford-on-Avon, where plots have been established to supplement trials previously carried out or in progress at Long Ashton. At these stations the Institute is working in close collaboration with the East Malling Research Station.

The establishment of the Technical Information Section under Mr. Swan, mentioned in the 1950 Report, has been in full working order during 1951, and has greatly improved the service that the Institute is able to provide to organised parties and visitors who wish to visit the Station. It is hoped that, with the further development of the activities of the section, it will be possible further to extend facilities to enable members of the Institute and fruit-growers to keep in close touch with the work in progress.

The lectures and practical work for students taking the University Diploma course in Horticultural Science were continued throughout the year, and will be carried on during 1952. In addition, ten members of the British Council Course No. 110 on Insecticides and Weedkillers attended the Station for lectures and demonstrations organised by Dr. J. T. Martin and given by several members of staff, on June 28th, 29th and 30th.

Lord Rothschild, G.M., Chairman of the Agricultural Research Council, visited the Station on January 31st to meet members of staff and to discuss in detail certain sections of the work with the staff concerned. The occasion also afforded an opportunity for him to meet the Vice-Chancellor and the Chairman and some of the University members of the Agricultural Committee. The visit was greatly appreciated both by the University representatives and the staff.

Sir John Simonsen, Chairman of the A.R.C. Conference on Cider and Fruit Juices, also paid a visit on October 30th to discuss the work of the Cider and Fruit Juices Section. The visit was a most profitable one for the members of the section, and the thanks of the Institute are due to Sir John for his help and interest in this work.

The Station was also fortunate in welcoming Professor R. G. Baskett, Agricultural Attaché to the British Embassy, Washington, on October 8th, during his period of leave. The Institute is kept in close touch with agricultural research in the U.S.A. through Professor Baskett's office, and it was a pleasure to have an opportunity of discussing with him many problems of mutual interest.

The Governing Body and members of the Institute and the members of the Agricultural Committee will all wish to record in this report the award of the C.B.E. in the New Year's Honours to Emeritus Professor B. T. P. Barker and to offer him their heartiest congratulations. All connected with the Institute know how fitting it is that his long and valuable services to the Institute should have been officially recognised in this way.

The Cider Advisory Committee and its sub-committee on Cider Making met during the year, but no meeting of the Orcharding sub-committee was held as there were no items of business for discussion.

The main committee approved a scheme for the training of cider judges, as the number of competent judges to cover the competitions held at Shows and Exhibitions is at present very small. The scheme is now in operation and consists essentially of two meetings each year at Long Ashton of the training panel, when those under training are given experience on the characters of new and conditioned ciders.

The committee also recommended to the Management Committee of the Institute that the basis for the nomination of candidates for the annual Memorial Studentship in cider-making should be widened to include others besides the sons of farmers or farm-workers. The recommendation was subsequently approved by the Members of the Institute at their meeting on July 10th, and, in consequence, the studentship is now open to workers in the counties of Devon, Gloucester, Hereford, Monmouth, Somerset and Worcester. Mr. W. H. Evans, the 1950 holder of the studentship, completed his training on April 14th, 1951. No award was made in 1951 because of lack of candidates. It is hoped that the amended regulations will remedy this position.

In connection with the scheme for the establishment of new Cider Variety Trial Plots, reported upon in the 1950 Annual Report, four of the approved bush tree plots have now been planted with " maiden " trees ; their location is as follows :—

Hereford : Man-of-Ross, Ltd., Upper Marston, Peterstow, Ross-on-Wye.
E. B. & H. Thompson, Pixley Gate, Ledbury.
Messrs. Wm. Evans, Swanstone Court, Little Dilwyn, Hereford.
Essex : Messrs. Carter & Blewitt, Boxted, Essex.

Each plot comprises 120 trees, consisting of six varieties, on two root-stocks, viz. Seedling Crab and Crab " C." The varieties are as follows :—

Sweet Alford, Tremlett's Bitter, Yarlington Mill, Sweet Coppin, Ashton Brown Jersey and Medaille d'Or.

In connection with the plots for standard trees a further site has been approved in Hereford for 1954 55—the Hereford Farm Institute.

It is with regret that we record the death of Mr. W. D. McCreath, Director of the Quantock Vale Cider Company and one of our oldest members, and of Mr. G. Pallett, a Director of the Taunton Cider Company and a former representative of the National Association of Cider Makers on the A.R.C. Committee for research on Cider and Fruit Juices.

By the death of Mr. McCreath the last direct link between cider research at Long Ashton and the initial experiments at Butleigh Court, Somerset, has been broken. As a young chemist, working under the direction of the late Mr. E. J. Lloyd, F.I.C., F.C.S., consulting chemist of the Bath & West & Southern Counties Society, he was installed at Butleigh to carry out the now historic experiments on cider-making initiated by the Society with the co-operation of the late Mr. R. Neville Grenville. The seasonal work carried on there led, in 1903, to the establishment of a permanent research centre, the National Fruit and Cider Institute (now the present Research Station), at Long Ashton, to which the Butleigh activities were transferred. McCreath then turned to commercial cider production at The Quantock Vale Cider Co., Ltd., North Petherton. Before long he had brought that firm to the fore-front in cider competitions at the leading shows, and as recently as 1949 was awarded several first prizes at all the premier shows with two outstanding ciders, a sweet and a dry, which were unanimously regarded by the contemporary judges as the best for a generation. He attached the greatest importance to cider competitions as a means both of raising the standard of quality and of popularising the beverage. With these objectives in view he provided a challenge cup, the McCreath Cup, to be awarded to the cider-maker achieving the best results in open competition over a three-year period. One of the original Members of the Institute, he continued to support it wholeheartedly, giving the successive members of its cider staff the benefit of his long practical experience and availing himself freely of their co-operation in dealing with problems of mutual interest. He welcomed their visits to his factory, and the stimulating discussions on such occasions never failed to provide fruitful ideas for further investigations. His constant aim was to bring cider to the level of a high-grade wine in general quality of aroma and flavour, notwithstanding the normal difference in alcoholic strength. Both the cider industry and the Research Station owe much to his efforts.

Mr. R. G. W. Bush, who had been the representative of the Ministry of Agriculture and Fisheries on the Agricultural Committee and a member of the Finance and Business Sub-committee since 1930, resigned from these committees on 18th July on leaving to take up residence in Jamaica. The best wishes of the members of the Agricultural Committee and of the members of the Institute go with Mr. Bush on his retirement. His valued advice and friendship will be missed by his many friends at committee meetings and station gatherings.

We are pleased to be able to report the full restoration to health of Captain Andrew Haggard, who was able once more to resume his duties as a member of the Finance and Business Sub-Committee on November 8th.

Mr. Woodall has once more been undergoing hospital treatment, but we are glad tc report that he is now on the mend and likely soon to be back among us.

Staff.

There have been a number of important changes in the scientific and administrative staff during the year, the most notable of which were the appointment of Dr. J. T. Martin as head of the section of Insecticides and Fungicides in succession to Dr. Hubert Martin, whose resignation was reported in the 1950 Report, and the resignation of Mrs. A. I. Edbrooke, Cashier, and Miss A. M. Evans, Director's Secretary.

Dr. Martin comes to us after long experience in the field of insecticides and fungicides. After graduating, with biochemistry as chief subject, in the University of Birmingham in 1929, he joined the staff of the Rothamsted Experimental Station, where he worked in the Department of Insecticides and Fungicides under Dr. F. Tattersfield. During that time his work was chiefly concerned with derris and pyrethrum, to the chemistry of which he made important contributions. From 1942 until he came to Long Ashton he was on the staff of the Ministry of Agriculture and Fisheries' Plant Pathology Laboratory, Harpenden, in which capacity he served on many important inter-departmental committees dealing with insecticides and fungicides. He was also a member of the recent Working Party of the Ministry of Agriculture on Precautionary Measures against Toxic Chemicals used in Agriculture, and he spent the period April–July, 1950, in U.S.A. and Canada, investigating regulating measures and research in progress on insecticides and fungicides and weed-killers by Federal Authorities, Universities and commercial organisations. He has also served as chairman of the Plant Pests and Diseases Committee of the Association of Applied Biologists, and as a member of the Executive Committee and Secretary of the Insecticides Section of the 2nd International Congress of Crop Protection, held in London in 1949. He worked closely with Dr. Hubert Martin on committees for many years, so that he was well prepared to take over the duties of the post and to ensure the continuation of the work in the section without any serious break.

Mrs. A. I. Edbrooke joined the Institute staff as Director's Secretary in April, 1918, and from 1920 until her resignation on 30th June, 1951, she filled the post of cashier. We regret very much that illness at home forced her to resign from her post a year or so before reaching retiring age. Mrs. Edbrooke for many years had been a key worker in the Station administration. She had witnessed the continuous growth of the Station's work, with its increasing financial problems, and by her organising ability and infinite patience in dealing with people and problems, she played a great part in keeping the Station finances on a sound footing. She was well known to many of the older members of the Institute and to cider customers and the business representatives of the many firms having connections with the Station. She was popular with her colleagues and regarded by all as a source of never failing help for their more material needs. The older members of staff also recall the part she played prior to the war in the social life of the Station, and her readiness to help on festive occasions. Her departure has left a gap that will not easily be filled in the office and in our Station community. We wish her every happiness in her retirement, and hope she may join us on social occasions for many years to come.

Miss Evans first came to Long Ashton in 1915, as Secretary to the Livestock Officer of the Ministry of Agriculture, who at that time had his office at the Station, and during that time she also acted as Secretary to the

Gloucestershire Old Spots Pig Society ; in 1920 she joined the Institute staff as Director's Secretary. From 1924, when the post of Deputy Director was established, she undertook the dual role of Directors' Secretary and continued as such until 1943, when the present Director was appointed to succeed Professor Barker and the post of Deputy Director was terminated. From 1943 until her resignation she continued to serve as Director's Secretary. The posts occupied by Miss Evans carried very heavy responsibilities. Not only was she responsible for the business correspondence of the Directors but she also dealt with academic and technical matters with officers of Government Departments and the University, and attended to arrangements for the reception of organised parties and visitors to the Station. She also assumed responsibility for the typing of scientific papers and technical reports, and the preparation of the minutes of many of the Station Committees, and the reception of scientific visitors to the Station. But her activities did not cease with the carrying out of her official duties. She was intensely interested in all matters relating to the staff and the welfare of the Institute, and this led her to undertake all sorts of work to these ends. There is perhaps no member of staff who has not received some kind of help from her, and her extra labours often resulted in her spending long hours in her office in the evening. Nor did her efforts to help others cease with the staff and committee members ; members of the Institute and innumerable visitors have all had cause to thank her for help with their varied problems and for her genial hospitality. In particular, the two Directors whom she served would wish to place on record their appreciation of her valuable work for the Institute and their personal thanks for her ever ready and untiring help throughout her long period of service.

It is a great pleasure to all her friends and colleagues to know that she resigned her appointment to marry an old member of our staff, Dr. D. A. Osmond, now stationed at the Rothamsted Station, and our best wishes for their future happiness go with them.

Appointments : Dr. J. T. Martin was appointed as Head of the Section of Insecticides and Fungicides from 1st March, 1951.

Mr. J. D. Phillips was appointed as Biochemist in the Cider & Fruit Juices Section from 1st August, 1951. Mr. Phillips graduated in the recently established Honours School of Biological Chemistry in Bristol University in June, 1951. He served as an officer in the R.E.M.E. from 1943 to 1947 in Scotland, West Africa, Syria, Egypt, Cyrenaica and Palestine, where, with the rank of Captain, he was in charge of the wireless workshop at Haifa for nearly three years.

Mr. J. G. Carr was appointed as Microbiologist to succeed Mr. R. C. Codner in the Cider and Fruit Juices Section from 13th August, 1951. Mr. Carr is also a graduate of Bristol University, and, prior to doing his National Service, had served as an Assistant at Long Ashton in the Plant Nutrition Section in 1945-46.

Mr. D. L. Abbott was appointed as Pomologist from 1st September, 1951. Mr. Abbott is a graduate of London University, having pursued his undergraduate studies at University College. He served in the Royal Engineers from 1939 to 1946, taking part in the D-Day landings and serving thereafter, with the rank of Captain, in various European countries. He was appointed Captain Garrison Engineer in 1945.

Miss Margaret Johnstone was appointed as Microbiologist in succession to Miss L. D. Kendall in the Domestic Preservation Section from 1st October, 1951. Miss Johnstone is a graduate in Domestic Science of the University of London, and received her training at the King's College of Household and Social Science.

Mr. E. Somers was appointed as Physical Chemist for special work on Spray Retention on Foliage under a special grant of the Agricultural Research Council, from 15th October, 1951. Mr. Somers is a graduate of Leeds University, and, since graduating, had spent some time in an industrial post.

Appointments to and promotions of assistant staff were as follows :—

Miss B. A. Rake, Assistant Experimental Officer, Pomology, from 25th April, 1951. Miss Rake is a graduate in Horticulture of Reading University, and has had previous experience on the staffs of the Yorkshire Institute of Agriculture, East Malling Research Station and Wye College, Kent.

Mr. C. W. Harper, Assistant Experimental Officer, was appointed to the post of Recorder, Spraying Experiments, from 22nd October, 1951. Mr. Harper had been on the staff of the Station for two years before the war, and, in addition, he has had experience of fruit farming in New Zealand and in Somerset and Devon.

Mr. H. M. Webber, promoted from Assistant (Scientific) to Assistant Experimental Officer, Plant Nutrition, from 1st October, 1951.

Miss M. Hallett, promoted from Assistant (Scientific) to Assistant Experimental Officer, Plant Nutrition, from 1st October, 1951.

Mr. J. H. Llewellyn, promoted from Assistant (Scientific) to Senior Assistant (Scientific) from 1st October, 1951.

Mr. J. H. Russell, Assistant (Scientific), Entomology, from 1st October, 1950.

Miss A. J. Beagles, Assistant (Scientific), Pomology, from 16th April, 1951.

Mr. E. R. Wilkins, promoted from Laboratory Attendant to Assistant (Scientific) from 1st October, 1951.

Resignations : There were three resignations from the scientific staff during the year.

Mr. R. C. Codner resigned from 28th February, 1951, to take up an appointment with the Medical Research Council at the Anti-biotics Research Station, Clevedon, Somerset.

Miss L. D. Kendall resigned from 31st August, 1951, to take up a post with Messrs. Brand's, food manufacturers, in London.

Mr. J. D. Cuthbertson resigned from 31st August, 1951, to study as a candidate for the Church.

Administrative Staff.

Appointments and Promotions : Following the resignations of Miss A. M. Evans and Mrs. A. E. Edbrooke, the following appointment and promotions were made :—

Miss M. H. Simpson was appointed as Director's Secretary from 20th August, 1951. Miss Simpson has previously held a number of commercial appointments, and from 1946 to 1951 had been Secretary of the Haberdashers' Aske's School for Girls, West Acton, London.

Mr. D. H. Pittman was promoted from Senior Clerk to Cashier from 1st August, 1951.

Mr. J. Packer was promoted from Ledger Clerk to Senior Clerk from 1st August, 1951.

Resignations : Mrs. A. I. Edbrooke, Cashier, resigned, from 30th June, 1951.

Miss A. M. Evans, Director's Secretary, resigned from 30th June, 1951.

Temporary Workers.

The following have been accommodated in the Institute laboratories during the year :—

Dr. H. Gleen of the Agricultural Research Council's team of workers on soil metabolism, continued his work throughout the year in collaboration with the Plant Nutrition Section.

Miss H. M. Ferres continued her work under an Agricultural Research Council grant for the second year in the Pomology Section.

Miss N. F. Millis concluded her tenure of the Boots' Research Scholarship in the Cider Section on 31st August, 1951 : she presented her Thesis for the degree of Doctor of Philosophy in September, and was awarded the degree in December.

Miss P. Miles of the N.A.A.S. continued her investigations of bulb nutrition under Dr. Hewitt throughout the year.

Mr. S. T. C. Wright, B.Sc. (Hons.), Reading, was appointed to the Boots' Scholarship from 1st September, 1951. Mr. Wright, who graduated in Agricultural Botany in 1951, is to study under Dr. Luckwill in the Pomology Section.

Visits Abroad.

Mr. S. H. Crowdy completed his period of study-leave at the Rhode Island Agricultural College, U.S.A., in June, and returned to the Institute on July 14th. Since his return Mr. Crowdy has submitted a report to the Agricultural Research Council on the investigations he carried out on plant chemotherapy at the Rhode Island Station in collaboration with Dr. F. L. Howard.

Mr. S. H. Bennett attended the IX International Congress of Entomology in Amsterdam in August, and after the Congress he visited several laboratories and experimental stations in Holland concerned with problems of the fruit-growing industry.

Miss Margaret Sturdy visited Cornell University's Department of Home Economics, the New York Agricultural Experimental Station, Geneva, and the U.S.D.A. Bureau of Human Nutrition and Home Economics, Beltsville, Washington, with the aid of a grant from the Agricultural Research Council, whilst on a private visit to U.S.A. Miss Sturdy subsequently prepared a report on her visit which, at the request of the Ministry of Agriculture, is to be widely circulated among County Domestic Science Instructresses and Women's Institutes.

The Director visited Spain in May as the guest of the Consejo Superior de Investigaciones Cientificas, to give a number of lectures and to visit centres of agricultural research in Madrid, Zaragoza, Valencia and Granada. He also attended the 2nd International Fertiliser Congress in Rome in October at the invitation of the Congress Committee, and submitted two reports on behalf of the United Kingdom delegation.

Buildings and Land.

Some progress has been made in connection with the development scheme for buildings mentioned in the 1950 Report. A memorandum outlining the needs of the Station for buildings and greenhouses and making proposals, with priorities, for the various items was submitted to the Agricultural Research Council, together with relevant plans and drawings, in February. Discussions with members of the Council staff have subsequently taken place concerning the proposals for a greenhouse unit included in the scheme and detailed drawings, embodying the points raised, have been prepared. A letter was also received from the Ministry of Agriculture in August authorising the preparation of detailed plans for the items under Priority A of the building scheme. These comprise a store for the Biology Laboratory to be used as temporary laboratories, additional lavatories in the main laboratory building, extension of the preparation room in the main laboratory building, and farm sheds to replace dilapidated sheds standing on the site of the proposed new laboratories. The estimated cost of these items is £3,500. The necessary arrangements to proceed with the work are in hand, and it is hoped to carry out the building during 1952. The provision of these items will merely alleviate the present serious congestion and will enable progress to be made on certain lines of work which are at present completely held up. No substantial easement of the congestion can be effected until the larger items of the building scheme are completed.

During the year considerable repairs were carried out on the floors and roof of the cider factory, and the Home Kitchen Hut and the Carpenters' Shop Block were re-roofed. Good progress was made in the erection of a range of pre-fabricated implement sheds and an extension to the fruit store. These were urgently needed as, with the present accommodation, many implements and machines cannot be put under cover and the fruit store is too small to contain the orchard boxes.

Fenswood Farm House has been re-wired and re-decorated to accommodate new tenants.

On the farm a stage has been reached when a considerable amount of clearing and thinning of the older experimental areas is necessary, and new plantings are needed for future experiments.

For the winter of 1951/52 a large programme of these operations was planned, and considerable progress has been made. The main items concerned are as follows :—

Clearing and thinning : *Plot* 10. Removal of standard trees from the plot, leaving a plant of bush trees at 30 ft.

Plot 11. Apple variety plot, now used for miscellaneous small experiments. Removal of alternate rows, leaving rows at 30 × 15 ft., suitable for experiments on automatic spraying methods.

Plot 20. Cider bush plot of 9 acres. Removal of alternate rows, comprising the trees of the four test varieties on M.I. rootstock and leaving the trees on Seedling Crab at 40 × 20 ft. The trees at 20 × 20 ft. had become very crowded under the cultural treatments favourable to growth.

Plot 21. Apple rootstock experiment, testing rootstocks raised at Long Ashton for standard trees. This plot of 6 acres has been cleared of trees on the completion of the experiment to prepare it for experiments on blackcurrants.

Plot 23. Apple pathology plot. Half-standard trees of Bramley's Seedling to be removed as a necessary thinning operation.

Planting : *Plot* 5. 300 seedling cider trees raised from crosses to be close-planted for making preliminary selections.

Plot 6. A number of seedling apples and pears raised from crosses to be planted for preliminary selections for dessert quality.

Plot 13. Dwarf pyramid apple plot. It is proposed eventually to plant out 5,000 trees for use in pilot experiments by the Pomology and Pathology Sections. 250 trees of Worcester Pearmain will be planted in 1951/52 and the remainder in 1952 53.

Plot 18. Cultural and manurial experiment with dessert apples, 5 acres approximately, using varieties Cox's Orange Pippin and Worcester Pearmain, with Bowden's Seedling in the buffer rows. 530 maiden trees at 20 ft. to be planted.

Plot 30. New cider bush tree trial of recommended varieties, 8 acres approximately. Trees comprise a total of 324 trees of the varieties Court Royal, Yarlington Mill, Tremlett's Bitter, Stoke Red, Sweet Coppin and Foxwhelp. The planting has been completed. The site used for this plot is on the extreme south side of the farm, the soil being derived from Lower Lias clay.

The orchard is planned to allow of the use of automatic spraying.

Research Activities.

Plant nutrition :

Fruit Crops. Further pot experiments were made using radioactive phosphorus to study the effects of Perennial Rye-grass on the uptake of phosphate by Malling I rootstocks. Preliminary indications were that grass cover increased the uptake of fertiliser phosphorus.

Leaves of apple trees in grass showed a much higher phosphorus and potassium content and lower nitrogen and magnesium than those in clean cultivation or in annual cover crops.

Apple trees with less than 15 p.p.m. zinc or 4 p.p.m. copper (in dry matter) developed deficiency symptoms which were corrected by foliage sprays of 0·1% zinc sulphate and 0·5% copper sulphate respectively. Magnesium deficiency in apples occurring in 1951 was not corrected by subsoil injections of magnesium sulphate made in 1950 or by soil surface applications in 1951.

No conclusive results have yet been obtained from the use of 1% urea sprays on foliage of apple and pear.

In the strawberry plots on the long-term manurial trial, yield was depressed by nitrogen omission and drastically reduced by omission of potassium. Phosphorus omission showed no measurable effect on growth or yield.

Other Crops : *Molybdenum nutrition.* Studies of the role of molybdenum, with special reference to nitrate metabolism, showed that cauliflower grown with 0·00005 p.p.m. molybdenum at a high nitrate level developed typical "Whiptail" symptoms. Dry weight production increased rapidly up to 0·005 p.p.m. molybdenum, and thereafter slowly to the maximum. Ascorbic acid increased sharply up to 0·0005 p.p.m. molybdenum, but not appreciably beyond. Nitrate nitrogen accumulated at low molybdenum levels, reaching 17% of dry matter. There was no accumulation of nitrite, and amino-acids were reduced. Less glucose, fructose and sucrose were found in the absence of molybdenum. Tests with different sources of nitrogen indicated that molybdenum was essential irrespective of nitrogen source. High molybdenum was acutely toxic with urea and ammonium sulphate, less so with nitrite and ammonium nitrate, and not toxic with inorganic nitrate.

Evidence was obtained that molybdenum activates reducing systems. Reducing activity varied in proportion to the molybdenum status of plants, and its distribution in sections of cauliflower tissue, as indicated by triphenyl tetrazolium chloride, was identical with that of molybdenum. Deficiency of

molybdenum caused an accumulation of inorganic phosphate in tomato and cauliflower and a depression of ascorbic acid in many species.

Molybdenum deficiency markedly accentuated manganese toxicity in tomato.

Visual records of molybdenum deficiency symptoms were extended. For the first time these were seen on radish, carrot and potato (after two years). Large-seeded legumes proved relatively insensitive to molybdenum deficiency.

An experimental centre on a Lower Greensand soil (pH 5·0) was used for field trials on lettuce. Acute symptoms of molybdenum deficiency were seen on the untreated plots. Significant increases in yield followed the addition of lime at 3 tons or sodium molybdate at 2 and 4 lb. per acre. Treatment with gypsum reduced yield.

On an adjacent experimental plot of cauliflower, symptoms of " Whiptail " occurred on the controls and were enhanced where gypsum had been applied. Lime (6 tons per acre) or sodium molybdate (4 lb. per acre), almost eliminated " Whiptail " and gave high yields. Sodium molybdate applied to the seed bed furnished the plants with sufficient molybdenum to form a marketable curd.

The special soil acidity plots were continued at Failand, Somerset, the trials being confined to a number of Brassica crops, viz. ; cauliflower, marrow-stemmed kale, rape, Brussels sprouts and cabbage. The soil treatments comprised carbonate of lime, dolomite and gypsum, all plots receiving an N.P. fertiliser in addition. The following deficiencies and excesses were demonstrated : potassium deficiency on the limestone and dolomite plots : magnesium deficiency on the gypsum, lime and untreated plots ; manganese excess, molybdenum deficiency and phosphate deficiency (aluminium excess ?) on the untreated and gypsum plots.

Iron metabolism. A factorial experiment on potato using iron, potassium and phosphorus, showed that in potassium-deficient plants extra iron apparently restricted translocation of potassium from old to young leaves. High potassium with high iron and low phosphorus induced calcium-deficiency symptoms, including medullary necrosis of tubers. Extra potassium increased iron accumulation in tubers at the expense of roots ; extra iron and potassium increased phosphorus accumulation in tubers at the expense of roots and shoots.

Chromatographic examination showed a linear relationship between chlorophyll and carotene, which both responded to potassium in the same way.

In spinach beet foliage, chlorophyll magnesium was less than total acetone-soluble magnesium, which comprised only 3–10% of the total magnesium. Copper and zinc decreased total magnesium, copper and molybdenum decreased acetone-soluble magnesium, and copper, zinc, manganese and molybdenum decreased chlorophyll and chlorophyll magnesium.

In tomato plants grown at eight levels each of iron and manganese, growth was greatly increased by iron or manganese at the lower concentrations, and was linear for log. manganese increments up to 0·11 p.p.m. ; thence to 1 p.p.m. there was no change and at 5·5–27·5 p.p.m. a depression. Iron content did not increase in relation to iron increments. Chlorophyll content increased with increments of iron and manganese, more so with the former.

Other micronutrients. Tests with tomato, lettuce and sugar beet gave no evidence of the essentiality of cobalt, nickel and gallium for plant growth. Tomato, oat and lucerne were especially sensitive to copper deficiency. The large-seeded legumes were less sensitive.

On tomato, iron deficiency and direct injury effects were produced by nickel and cobalt, but zinc at equivalent dosages was less toxic. In the water extract from the tomato leaves, all the nickel, 60% cobalt and 40% zinc were present in bound form.

Aspergillus niger has been used for routine determinations of the availability of copper, zinc, manganese and molybdenum in soils and crop plants. Detailed biochemical and physiological investigations of the micronutrient requirements of *Aspergillus niger* were made.

Leaf analysis of oats from manurial trials suggested an early-season manganese content of 40 μg per g. in the mid-stem leaves as the threshold level for manganese deficiency.

Radio-tracer investigations. Strictly speaking, the use of the radio-active tracer method as a tool in plant nutrition studies requires the absence of any effects on plant metabolism that can be attributed to radio-activity. A two-years' study of these " radiation effects " has shown that they occur occasionally even at low radio-active levels, but that they are small and did not increase with the higher doses of radio-active phosphorus used. In general, such temporary checks are partly or wholly compensated by resultant stimulation of plant growth. It is concluded that the overall results of radiation effects, although significant, are not of sufficient magnitude to invalidate the method.

The effect of cobalt on the uptake of fertiliser phosphate by tomato was studied in pot experiments using orthophosphate labelled with P^{32}. Determinations so far made indicate that cobalt increased the total phosphorus content of the plants, reduced dry weight and induced iron deficiency by decreasing iron in the roots. (Further references to radio-tracer investigations are given under the headings " Fruit Crops " and " Pests and Diseases and their Control ").

Organic constituents of plants. Examination of chromatographic methods for free amino-acids showed that neither storage of leaves at 5° C., macerating in alcohol, nor delay in filtering affected amino-acid yield, indicating that enzyme modification of amino-acids was not a significant factor in the normal method of leaf extraction.

Using paper chromatography, changes in amino-acid composition have been followed in homogenates of tomato or cauliflower leaves added to M/100 solutions of various subtrates in phosphate buffer. After incubation at 37° C. for 20 minutes, transamination reactions occurred. These showed no gross response to different levels of manganese or molybdenum nutrition of the original plant material. However, the decarboxylation of glutamic acid in tomato is apparently enhanced at low manganese level.

Pomology and Plant Breeding.

The technique of paper chromatography has been used in conjunction with the oat coleoptile test to separate and identify the auxins and growth inhibitors in plant extracts.

Vitamin K_3 at 50 p.p.m. applied at the pink-bud stage increased fruit set in Edward VII apple, but not in Cox or Worcester.

In the substituted aliphatic acids, which have been synthesized and tested on tomato ovaries, it was found that increasing the length of the side chain diminished parthenocarpic activity. Work with a series of naphthyloxy aliphatic acids showed that physiological action was high in the compounds having an odd number of CH_2 groups.

The exposure to cold required by apple seeds to break their dormancy was reduced by a preliminary storage at about 15° C. above normal after-ripening temperatures. Determinations were made of the minimum periods at 5° C. necessary to break dormancy in buds of apple, pear, plum and black-currant varieties.

A pre-blossom application of maleic hydrazide delayed ripening of raspberries and strawberries, but not of apples and blackcurrants.

A report has been issued comparing the effects of modified-leader and open-centre tree shapes on growth and yield of five commercial apple varieties.

Final records have been made of the standard apples on the Long Ashton clone stocks, of which G8 is recommended.

A dessert apple produced from a cross between Worcester Pearmain and McIntosh Red is being included in the National Fruit Trials.

Growing-point dissections of strawberry plants showed that flower primordia are formed in July in Auchincruive Climax, but in September in Royal Sovereign and Perle de Prague.

Pests and Diseases and Their Control.

Investigations made jointly by the chemical, entomological and mycological sections extended the studies of the relations between chemical structure and biological activity of insecticides and fungicides. Further evidence was obtained that the electron-attracting capacity of the CCl_3 group is of major importance for the insecticidal activity of DDT.

A number of isosteres and analogues of DDT was synthesized and bio-assayed: a chlorphenyl chloronitropropane showed twice the toxicity of DDT to the granary weevil. In the parathion group paraoxon proved more toxic to locusts than parathion.

Over forty substituted naphthoxy acids were prepared and screened against Chocolate Spot of bean for systemic fungicidal activity. Systemic fungitoxicity was found to be widely distributed but no individual compound showed outstanding merit. Chlorphenyl ethanol compounds gave systemic fungicidal action of the same order as the corresponding oxyacetic acids.

In the investigations on systemic insecticides, tests with P^{32}-labelled Schradan showed that entry into the leaf and translocation both occur mainly during periods favouring photosynthesis. Translocation rapidly followed penetration, but affected only a small proportion of the total absorbed. The rate of breakdown of the insecticide in the plant was much higher in beans than in coleus or chrysanthemums.

Field tests with insecticides included further observations on the possibilities of spring sprays as substitutes for winter washes. DDT emulsion at the green-cluster stage was found satisfactory against aphids, capsids and caterpillars, but not apple sucker. Bio-assays, using apple sawfly larvae, were made by placing standardized doses of insecticide in the calyx cup of the apple.

Preliminary work on residues showed that there was little risk of excessive DDT spray deposits on harvested soft fruits when the normal recommendations for spraying were followed.

Fungicide field trials demonstrated a high level of apple scab control by zinc dimethyldithiocarbamate and by a phthalimide spray. Glyoxalidine was effective against scab on Comice pears in a large-scale trial.

An autumn application of phenyl mercury chloride reduced leaf scar infection by the apple canker fungus by restricting spore formation on existing cankers.

Further studies on brown rot indicated that fruit wounds more than a day old were resistant to infection. For winter eradicant applications pentachlorphenol preparations showed promise.

In the tree fruit virus investigations, a rasp-leaf disease was graft-transmitted from Merton Heart cherry to the Mazzard F 12 1 stock.

Work on the application of crop protectants included studies of methods of producing standardized isolated droplets, and further determinations of spray performance in relation to nozzle design. A low-pressure sprayer employing a gear pump was designed for spraying cordons, dwarf pyramids and bush fruit. A low-volume concentrate sprayer was constructed for the Colonial Office and delivered to the Locust Organization: seven light-weight manual sprayers were also supplied for mosquito control. A specification for a robust, easily-maintained stirrup pump was prepared for the World Health Organization.

Cider and Fruit Juices.

Raw materials of cider and apple juice. Studies of methods of orchard sampling showed larger variations between the composition of fruit from neighbouring trees than between individual samples from the same tree. A random sample of 40–80 fruits per tree appeared satisfactory.

In the long-term study of the effects of cover-crop treatments on vintage quality of cider fruit, trees in perennial rye grass have given fruit with a significantly higher juice acidity and lower nitrogen content. This, in one year, was associated with a slower rate of fermentation. In these trials, the specific gravity of the juice has not so far been significantly affected by cultural treatment or by rootstock (Malling I compared with Seedling Crab).

In tests of deferred pressing, juices of the culinary varieties tested showed a marked fall in acidity when the fruit had been stored for some months. Up to March there was little deterioration in juice quality and no increase in storage deposit in bottle. Low-acid (e.g. dessert) varieties might present processing difficulties after much briefer periods of storage. Fruit of Dabinett could be stored for four months before pressing without adverse effect on the quality of the resulting cider.

Processing operations (cider and apple juice). Comparisons under conditions of normal usage showed little advantage of wicker press-racks over wooden racks. In investigations on the maintenance of pectin content in pomace it was found that pectin de-methylation could occur in the wet pomace of certain apple varieties.

The amounts of copper, zinc, iron, lead and arsenic in unsprayed apples and their juices and cider were estimated and the use of paper chromatography in this work was explored. Paper chromatography was also employed to separate amino-acids in juices and in the corresponding ciders: this showed that fermentation was accompanied by a fall in soluble nitrogen and amino-acids.

Heat inactivation of the pectin esterase in apple juice was proved to be markedly affected by pH. This finding is of importance in apple juice pasteurisation.

In studies of juice treatment prior to fermentation, it was found that defecation of the juice was accompanied by a removal of pectin and nitrogen followed by a slower rate of fermentation.

The acidity of juices from culinary apples was reduced by ion-exchange treatment : on a laboratory scale this treatment was applied without any marked deterioration in juice quality.

In the investigations of the chemical changes accompanying fermentation, paper chromatography was utilised in the separation of the organic acids in juices and ciders. The factor limiting yeast growth and fermentation in some naturally sweet ciders was found to be a double deficiency of aneurin and nitrogen. Further work has been done on fermentations by selected yeasts using both chemical and micro-biological tests.

The disorders of cider were investigated by isolating and characterising the bacterial flora, with special reference to the species giving rise to " sickness " and " ropiness " and to those responsible for malo-lactic fermentations.

A comparison was made of methods for the small-scale preparation of cider vinegars. The initial acidity of the medium appeared to be a factor of importance.

The pasteurisation of apple juice and cider was studied by following the temperature changes and their effect on the quality of the respective products.

The fermentation of reconstituted apple juice prepared from concentrates was investigated by comparing the effects of different methods on fermentation and on the quality of the product. Inoculation of the diluted concentrate with selected yeast cultures appeared to be beneficial.

Soft fruits and their products. The effects of manurial treatments on the quality of strawberries were investigated, attention being directed to crop yield, flavour, and content of ascorbic acid, sugar, acid and pectin. These factors were significantly influenced by manurial treatment : the potassium status of the plants was shown to be of major importance in the maintenance of quality on a site where potassium deficiency is acute.

Domestic Preservation of Fruit and Vegetables.

Heat penetration in the bottling and canning of fruits and vegetables has been plotted for a range of methods and varieties. The minimum times and temperatures required to inactivate the oxidase enzyme systems of fruit have been determined.

The preserving quality of 28 varieties of fruit and 11 of vegetables has been assessed by bottling, canning and deep-freezing.

Other Activities.

Members' Day. The Annual Field Day for members and the Annual General Meetings of Members and Governors of the National Fruit and Cider Institute were held on July 10th, 1951.

As usual, a programme illustrating important investigations in progress and practical aspects of fruit growing was carried out. The main items were as follows :—

1. *Field plots.* New methods of spraying ; newer fungicides and insecticides ; systems of planting, tree building and pruning.
2. *Pot culture exhibits.* Relation of nitrogen supply to molybdenum requirements in cauliflower ; copper, zinc and molybdenum deficiencies ; organic acid metabolism in potato.
3. *Cider house exhibits.* Cider apple varieties recommended for planting ; colour photographs and specimens of fruits of recommended varieties ; crop records of varieties from trial bush and standard orchards ; present distribution of cider orchards ; apple juice manufacture.
4. *Laboratory exhibits.* (a) Main Laboratories : Chemical and biological methods of diagnosing the mineral status of crops ; radioactive tracer technique for investigating fertiliser

problems ; preservation tests on fruit varieties ; plant hormones ; control of fruit drop ; blossom thinning sprays ; methods of estimation. (b) Biology Laboratories : Systemic insecticides.

Cider Sampling Day. The Annual Sampling Day was held on May 3rd, the morning being devoted to sampling ciders of the 1950 season. The year had been marked by a high rainfall and had been the second of two years of heavy crop ; the specific gravities of the juices were, on the whole, lower than in 1949. The ciders of the first group offered for tasting were made from single varieties of fruit recommended for planting and from two lesser-known varieties chosen from those sent in for vintage trial. The second group of ciders demonstrated the effects of pre-fermentation treatments of the juice and of fermentation by selected yeasts. The differences in vintage quality exhibited by these ciders were considered to be small and no clearly defined preference was apparent. In the previous season the ciders fermented by a wine yeast had been voted the best.

The third group of samples offered a comparison between ciders which had been sweetened by the addition of sugar after fermentation and others in which the natural sugar had been retained. The fermentation of these latter ciders had been restricted by first subjecting the juice to a defecation or "keeving" process. Other ciders shown illustrated some of the uses of concentrate in cider-making. An exhibit in the cider house showed some of the other work of the cider and fruit juices section by means of charts and samples of ciders and apple juices.

During the afternoon demonstrations were given in the plantations of methods of tree pruning and tree building and of ground crop spraying.

Visitors.

Forty-seven organised parties visited the Station during the year, six less than in each of the previous two years. The parties included the N.F.U. Fruit Growers' Annual Tour, party from The British Council Course on Insecticides and Weedkillers, N.A.A.S. Advisory Chemists, Horticultural Officers' Course and Agricultural Officers, Festival of Britain Parties, British Mycological Society and Society of Chemical Industry's Agriculture and Food Groups' Tour. The full list is as follows :—

Bristol Grammar School.
British Council Course on Insecticides and Weedkillers.
British Mycological Society.
Calne and District Allotment Holders' Association.
College of Technology, Bristol, Pharmacy Students' Association.
Cossham Medical Society.
Diocesan Training College, Barrow Gurney, Bristol.
Dr. Morgan's School, Bridgwater.
Exeter and District Young Farmers' Club.
Festival of Britain Visitors.
Fishponds (Lodge Causeway) Townswomen's Guild, Bristol.
Guild of Insurance Officials, Bristol Branch.
Hallen and Henbury Women's Institute, Bristol.
His Majesty's Stationery Office, Bristol Branch.
Horfield Women's Guild, Bristol.
Keynsham Gardeners' Association, Somerset.
Knowle Townswomen's Guild, Bristol.
Long Ashton village visitors.
Mardon, Son & Hall, Ltd., Members of Caxton Works Trades Association Chapel, Bristol.
N.A.A.S. Advisory Chemists.

N.A.A.S. Horticultural Officers' Pruning Course.
N.A.A.S. Horticultural Officers' Refresher Course.
N.A.A.S. Officers, General Agriculture Section.
N.F.U. Fruit Tour.
Pill and Easton-in-Gordano Women's Institute, Bristol.
Ross Grammar School, Parents' and Teachers' Association.
Sea Mills Townswomen's Guild, Bristol.
Shirehampton Baptist Men's Contact Club, Bristol.
Society of Chemical Industry, Agriculture and Food Groups
Somerset County Council Rural Science Organisers.
Somerset Farm Institute, Cannington, nr. Bridgwater.
Special Schools Teachers' Association, Bristol.
St. Anne's Men's Contact Club, Bristol.
University College of North Wales, Department of Agricultural Botany.
University College of Swansea, Department of Biology.
University College of the South-West, Department of Botany and Horticulture.
University of Bristol Botanical Society.
University of Bristol, Department of Bacteriology.
University of Bristol Students' Chemical Society.
University of Nottingham, Department of Horticulture, Sutton Bonington, Notts.
Waterperry Horticultural School, Wheatley, Oxford.
West of England Association of Science Teachers.
West Rambling Club, Fishponds, Bristol.
Westbury Park Townswomen's Guild, Bristol.
Weston-super-Mare Grammar School for Girls, Somerset.
Wye College, Kent, Department of Horticulture.
Wyke Regis Women's Institute Produce Guild.

The Institute received many scientists from overseas, a number of whom worked in the laboratories for short periods. Among these visitors were the following :—

Visitors from Overseas (1951)

Australia : Dr. D. W. A. Stewart, Manjimup, Western Australia.
Dr. L. P. H. Jones, Melbourne.
Mr. R. W. Kerr, Commonwealth Scientific and Industrial Research Organisation, Canberra.
Mr. T. W. Hogen, Senior Entomologist, Department of Agriculture, Victoria.
Mr. C. W. Strutt, Agricultural Adviser, U.K. Trade Commissioner, Australia.
Mr. L. A. Thomas, Senior Research Officer, C.S.I.R.O., Stanthorpe, Queensland.

Canada : Mr. R. Barrett, Technocracy Incorporated, Windsor, Ontario.

Ceylon : Dr. E. J. Risdon, Chemist, Ceylon Rubber Research Scheme.
Mr. D. H. Constable, Agronomist, Ceylon Rubber Research Scheme.

Denmark : Mr. Hans Gumpel, Landbrugskandidat, Statens Forsegestation, Blangstedgaard.
Mr. Svend Jakobsen, Landbrugets Jorddyrkning, Landbohojskolen, Kobenhavn.
Mr. E. J. Norgaard Pedersen, Landbrugets Jorddyrkning, Rolighedsvej 23, Kobenhavn.
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Mr. Ernest Wolff, University of Florida.
Dr. W. E. McCauley, Product Development Director, Julius Hyman & Co., Denver, Colorado.

West Africa : Mr. J. F. Archibald, Cacao Research Institute, Tafo.
Dr. H. R. Mapother, Cacao Research Institute, Tafo.
Mr. D. Rhind, Secretary for Agricultural and Forestry Research, West African Inter-territorial Secretariat.

Six Colonial Journalists from Hong Kong, Malaya, Mauritius and Singapore.

*Members of the section of the British Council Course No. 110 on Insecticides and Weedkillers.

Shows and Demonstrations. Exhibits were staged at the following shows :—

Festival of Britain, South Bank Exhibition—May 3rd–September 30th.
Royal Horticultural Society—Chelsea, May 23rd–25th.
Bristol Seventh Annual Horticultural Show—August 29th–31st.
Bath and West Show—Dorchester, May 30th–June 2nd.
Three Counties Show—Gloucester, June 12th–14th.
Royal Agricultural Show—Cambridge, July 3rd–6th.

Students and Voluntary Workers. It is regretted that the number of research students must remain severely limited owing to lack of accommodation. Numerous applications for places continue to be received, but these unfortunately must be refused until new laboratories are available.

Dr. S. C. Agarwala continued his work as a Ph.D. student in the Plant Nutrition Section under Dr. Hewitt.

Mr. E. J. Skerrett continued his work as a Ph.D. student in the section of Organic Chemistry under Dr. Woodcock.

Mr. C. G. Guttridge, a Ph.D. student of Reading University, worked at the Institute from 26th February, 1951.

Miss D. M. Payne continued to work as a voluntary worker in entomology and photography under Dr. Kearns.

Mr W. H. Evans, holder of the Memorial Studentship in cider-making, completed his six months period of training on 14th April.

Mr. J. Pullin, Compton Greenfield, and Mr. A. Pullin, Falfield, completed their courses in cider-making on 2nd and 16th October respectively.

The following also spent short periods working in the laboratories or plantations :—

In the Plant Nutrition Section, with Dr. Hewitt and Dr. Nicholas : Dr. Werner Gruppe, Institut für Obstbau und Baumschule, Hannover ; Mr. D. V. K. Rao, Indian Government Officer.

In the Plant Nutrition Section, with Dr. Nicholas : Dr. R. A. Webb of the Medical Research Council, prior to service in West Africa ; Mr. S. I. Jakobsen and Mr. E. J. N. Pedersen, Landbrugets Jorddyrkning, Copenhagen ; Dr. Valentin Hernando, Instituto de Edafologia y Fisiologia Vegetal, Madrid ; Mr. M. Neenan, Department of Agriculture, Wexford, Eire.

In the Cider and Fruit Juices Section, with Dr. Pollard and Miss Kieser : Mr. Hans Gumpel, Landbrugskandidat, Statens Forsegestation, Blangstedgaard, Denmark.

The following courses were given in the Domestic Fruit and Vegetable Preservation Section :—

Preservation and Judging Preserves—July 9th–27th.

Preservation of Fruit and Vegetables—August 27th–September 7th.

Thanks are due to Miss P. Leicester for assistance given in the courses.

Social Activities. The Welfare Committee continued to serve the Station during the year under the Chairmanship of Dr. Nicholas. As usual it organised the Christmas Party, which was held in the Village Hall on December 21st, when over 220 attended. The staff were very pleased to welcome back again Dr. and Mrs. Osmond on this occasion. A pantomime—" Ali Baba and the Forty Thieves "—which had been written and produced by members of the staff, was much enjoyed.

The Cricket Club continued under the difficulties mentioned last year, and again entered for the Bristol Evening Post Challenge Cup.

The Tennis Club again played a number of matches in the Bristol Tennis League. Hard and grass courts were well used throughout the season in spite of bad weather ; lunch-hour play continued with enthusiasm, and matches took place in the evenings.

Acknowledgments. Special acknowledgments are due to members of the staff who have carried out special duties in addition to their normal work. Dr. Kearns has continued to give valuable assistance in connection with the planning of new buildings and in organising and supervising the workshops, including repair work to equipment, machinery and tools for the laboratories and farm. Mr. Spinks continued to act as librarian. Dr. Nicholas was elected to the office of Chairman of the Welfare Committee and was responsible in this capacity for organising the Christmas Social. Miss Sturdy again acted as Chairman of the Canteen Committee, and in her absence during the latter

part of the year Miss James and Miss Moseley undertook catering for the Canteen. Best thanks are due to these and to other members of staff who have helped in various ways during the year.

Grateful acknowledgment is also made to all members of the assistant and technical staffs and to all employees for their loyal support in carrying out the programme of research work.

Thanks are also due to all outside friends, particularly to growers and commercial firms, who have co-operated in experiments or helped in other ways during the year.

TRIALS OF CLONAL APPLE ROOTSTOCKS SELECTED FROM "FREE" AND "CRAB" SEEDLINGS. IV. PERFORMANCE WHEN WORKED WITH FOUR SCION VARIETIES AND GROWN AS STANDARD TREES AT LONG ASHTON.

By G. T. SPINKS.

Introduction.

The origin of this series of apple rootstocks was described in 1931 (2). In 1940 Hoblyn (1) and Spinks (3) published the results of trials at East Malling and Long Ashton respectively and in 1946 (4) the present author described the results of trials at Long Ashton and at a number of other places in the West of England.

Since the publication of the results obtained up to 1944 no further records have been obtained except from two plots of standard trees at Long Ashton. At the end of 1951, as it seemed that these plots were unlikely to yield any additional information of value, the trees were grubbed. The present paper summarises the observations made on these two plots during the whole life of the trees.

Experimental Data.

The material used, the sites, the cultural treatment and the lay-out of the plots were described in the previous paper under the headings of Plot 21 and Plot 22. The two plots are adjacent and are similar in every way except that Plot 21 was under clean cultivation, with intercrops of blackcurrants, until 1944, whereas Plot 22 was an old pasture when the trees were planted and has been maintained as a grass orchard during the whole period of the trial.

Each plot was divided into two parts, the soil conditions in the lower section being good while those in the upper section were known to be rather inferior, and blocks of trees on each rootstock were randomized on the two parts. Each block consisted of four or five trees but in a few cases the blocks were not replicated. For this reason, and also because the records are not quite complete, mainly owing to war conditions, the results have not been statistically analysed and the figures given in the following tables show only the mean values.

Four scion varieties were included and each variety has been considered to form a separate trial. In the tables the mean total crop per tree for the whole period of the trial is shown, the rootstocks being arranged in order of weight of crop. In each table the mean girth of the trees at the end of 1951 is also given. Heights and spreads of the trees were measured but have not been tabulated as they usually follow the same order as the girth measurements : exceptions sometimes occur where heavy cropping has tended to reduce the height and increase the spread.

TABLE I.

WORCESTER PEARMAIN, STANDARDS, 1928–1951.

PLOT 21.

Rootstock	M XII	E 7	F 10	G 7	F 11	D 1
Mean total crop per tree (kg.) for 23 years.	558	315	256	156	132	103
Mean trunk girth (cm.) at 23 years.	68·9	45·8	41·1	40·0	38·1	28·8

TABLE II.

WORCESTER PEARMAIN, STANDARDS, 1928–1951.

(Trees 2 years old at planting).

PLOT 22.

Rootstock	G 8	E 7	F 11	G 7	G 6	F 10	F 2
Mean total crop per tree (kg.) for 23 years.	338	306	284	260	216	207	143
Mean trunk girth (cm.) at 23 years.	61·1	58·9	54·4	56·5	54·5	60·6	59·0

TABLE III.

WORCESTER PEARMAIN, STANDARDS, 1928–1951.

(Trees 5 years old at planting).

PLOT 22.

Rootstock	E 7	G 7	G 8	G 6	F 12
Mean total crop per tree (kg.) for 23 years.	405	378	360	254	99
Mean trunk girth (cm.) at 23 years.	58·5	64·1	58·9	58·6	57·0

TABLE IV.

BRAMLEY'S SEEDLING, STANDARDS, 1928–1951.

PLOT 21.

Rootstock	Hampshire Crab Seedlings.	G 8	Mixed Seedlings.
Mean total crop per tree (kg.) for 23 years.	604	562	549
Mean trunk girth (cm.) at 23 years.	77·8	76·4	82·9

TABLE V.

BRAMLEY'S SEEDLING, STANDARDS (Worked Low), 1928–1951.

PLOT 22.

Rootstock	G 8	E 7	F 11	G 7	F 10	G 6	F 2
Mean total crop per tree (kg.) for 22 years.	1,208	720	671	503	491	408	212
Mean trunk girth (cm.) at 23 years.	98·6	86·2	73·1	79·2	79·6	82·9	82·1

TABLE VI.

BRAMLEY'S SEEDLING, STANDARDS (Head-Worked), 1928–1951.

PLOT 22.

Rootstock	G 8	F 10	F 11	E 7	G 7	G 6	F 2
Mean total crop per tree (kg.) for 22 years.	805	721	564	484	446	341	268
Mean trunk girth (cm.) at 23 years.	73·4	83·6	70·0	67·6	71·2	66·0	59·1

TABLE VII.

NEWTON WONDER, STANDARDS, 1928–1951.

PLOT 21.

Rootstock	M XII	D 1	G 8	G 7	E 7	G 6	F 11
Mean total crop per tree (kg.) for 23 years.	530	497	492	464	359	209	198
Mean trunk girth (cm.) at 23 years.	89·2	72·1	73·6	65·9	65·7	58·4	46·0

TABLE VIII.

BLENHEIM ORANGE, STANDARDS, 1928–1951.

PLOT 21.

Rootstock	G 8	M XII	G 6	G 7	E 7	D 1	F 11	F 10	F 12
Mean total crop per tree (kg.) for 23 years.	220	219	209	190	157	124	115	107	83
Mean trunk girth (cm.) at 23 years.	88·5	95·1	87·7	57·9	60·0	72·4	44·5	73·4	60·2

Discussion.

The performance of the various rootstocks is considered under the headings of the different scion varieties.

Worcester Pearmain.

On Plot 21 much the heaviest crop was produced by the trees on M XII which were much larger than those on any other rootstock. Trees on D 1 were very small and bore the lightest crop. The order of crop weights corresponds exactly to the order of tree size and these results are similar to those obtained up to 1944.

On Plot 22 two sets of trees were planted at the same time but the trees in one set were three years older than those in the other at the time of planting. In the younger set of trees the relative performances of the stocks were almost unchanged, G 8 heading the list for crop weight and F 2 being associated with the lightest crop. Trees on F 2 and F 10 were higher in size order but still cropped poorly.

Among the older trees on Plot 22 the order of cropping and size was again almost unchanged. Trees on E 7 again headed the list for crop weight but it is doubtful whether the differences between the figures for E 7, G 7 and G 8 are significant. Rootstock F 12 is evidently associated with light crops.

Bramley's Seedling.

On Plot 21 only one clonal stock, G 8, was compared with two series of seedling stocks. Whereas up to 1944 the clonal stocks had produced slightly heavier crops, the figures for 1951 show that the Hampshire Crab Seedlings were giving the best crops, but it is doubtful whether the differences are significant.

On Plot 22 the records for crop weights do not include the year 1951, as in that year the crops on a large number of trees were ruined owing to a lack of control of aphis. The order of cropping of the low-worked trees up to 1950 was the same as in 1944 : stocks G 8 and F 2 produced much the heaviest and lightest crops respectively.

Trees on Plot 22 which had been formed by head-working the scion on rootstocks run up to standard height also showed little change in performance since 1944. The best crops were recorded on G 8 and the poorest on F 2, while the relative order of tree size remained almost unchanged. It has already been noted (4) that trees worked low have usually been larger and have cropped more heavily than head-worked trees and that the latter had a poorer root-hold, especially on stocks E 7 and G 6. Head-worked trees on F 10, however, have been larger and more prolific than low-worked trees.

Newton Wonder.

By 1951 trees on M XII, which had been slow in coming into bearing, were still much larger than any others and had also borne the heaviest total crop. The relative performances of other rootstocks showed little change and the smallest and least prolific trees were still those on F 11 and G 6.

Blenheim Orange.

This variety has borne very poor crops, the best having been produced by trees on G 8, M XII, G 6 and G 7 while F 12 is at the bottom of the list. The largest trees were those on M XII, G 8 and G 6 and the smallest on F 11.

General Conclusions.

Data obtained from these trials during the last seven years have confirmed the conclusions drawn at the end of 1944. Though no trial included a complete range of stocks it is possible to assess the performance of each stock by considering the results of each trial.

Rootstock M XII, when included in a trial, has always produced the largest trees and also the heaviest crop over a period of 23 years, though crops were small in the early years.

G 8 is near the head of every list for crop and size of tree, but its performance has not been quite consistent. Trees on E 7 and G 7 have always been moderately large and good croppers, while F 11 and G 6 are associated with smaller trees which have borne good crops considering the size of the trees.

Some stocks have been rather erratic in their performance, especially when worked with different scion varieties. This is the case with F 10, and particularly with D 1 which produced some of the largest trees of Newton but the smallest of Worcester. Rootstocks F 2 and F 12 have given consistently poor results.

All the above findings agree with those previously reported (4) from trials at various other centres. When the trees on Plot 21 were grubbed by means of a winch visual inspection of the roots revealed very little variation in the character of the root-systems and any small differences which were noted did not appear to be correlated with particular rootstocks.

If apple trees are to be grown in the form of standards it is desirable that they should grow rapidly to a good size, should have a good root-hold and should crop heavily over a long period of years, but cropping in the early years is not of so much importance.

Of the rootstocks tested in these trials M XII appears to be the most suitable for producing trees of the desired character. G 8, on the whole, seems to produce satisfactory standard trees but there is no conclusive evidence that it is superior to other vigorous stocks which are already fairly widely used. It is concluded that none of the other stocks in the trials can be recommended for standard trees, though F 11 and G 7 may be very satisfactory for growing bush trees and further trials are being carried out to test their value for that purpose.

Summary.

(1) Standard trees of four scion varieties worked on a series of clonal apple rootstocks have been grown on two trial plots at Long Ashton.

(2) A previous article recorded the results from these plots up to the year 1944 and the present paper includes data for a further seven years, which confirm the previous findings.

(3) Rootstock M XII was associated with the largest trees and the heaviest crops. Stock G 8 was generally satisfactory and may be of use as a stock for standard trees, but there is no evidence that it is superior to some other vigorous clonal stocks.

(4) It is concluded that stocks F 11 and G 7 are not suitable for standards but may be useful as stocks for bush trees. None of the other rootstocks can be recommended for general use.

Acknowledgment.

The writer wishes to thank Mr. G. E. Clothier, who has recorded all the data from the trials over a long period of years.

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(4) Spinks, G. T. (1946). Trials of clonal apple rootstocks selected from "free" and "Crab" seedlings. III Performance on a number of sites in the West of England when worked with several scion varieties. *Ann. Rep. Long Ashton Res. Sta.*, 1945, 19–43.

TRIALS WITH GROWTH SUBSTANCES AS FRUIT-THINNING AGENTS.

By L. C. LUCKWILL.

Introduction.

In those varieties of apple which habitually carry heavy crops in alternate years, thinning may be used to increase fruit size and quality, and to encourage fruit-bud formation in the "on" year with a view to inducing annual bearing. The high cost of hand thinning has led various workers, particularly in the United States, to investigate the possibility of thinning with chemical sprays. The principal types of thinning sprays which have been tested on apples are :—

(1) *Di-nitro materials*, such as sodium di-nitro-o-cresylate and di-nitro-o-cyclohexylphenol. Such materials have proved reasonably satisfactory on most American varieties of apple, but for the best results they have to be applied at full-bloom or shortly afterwards (1).

(2) *Growth-substances*, particularly 1-naphthalene acetic acid or its sodium salt. Applied at any time between late pink-bud and 3 to 4 weeks after petal-fall, at concentrations between 5 and 50 p.p.m. these substances will cause a substantial reduction in the crop of most apple varieties.

(3) *Oil-wax emulsions*, applied at full bloom may reduce fruit set by coating the stigmatic surfaces with wax, thereby interfering with pollination. In practice such sprays have not proved very successful.

(4) *Mixtures of polyethylene polysulphide and zinc dimethyl dithiocarbamate-cyclohexamine.* Such mixtures have been used primarily for scab control but in some instances have also given reduced fruit set. They have not yet been extensively tested as thinning agents.

Under English conditions it would appear that 1-naphthalene acetic acid (NAA) might have considerable advantages over other types of thinning agent, since, with this material thinning can be delayed until about three weeks after petal-fall. By this time danger of frost damage has largely passed, and the grower can assess with some certainty the initial set of fruit and the degree of thinning required. For this reason trials with this type of thinning agent have been carried out in 1950 and 1951. The 1950 trials were designed

primarily to investigate the mechanism of the thinning action, but incidentally information was obtained on the reactions of certain English apple varieties to this spray. This article deals with points of particular interest to fruit-growers, some of whom may wish to experiment for themselves with these sprays. A fuller account of these experiments is being published elsewhere (5).

Method.

In these experiments each spray treatment was applied to four to eight large limbs of mature bush trees, carrying between one and two thousand fruit clusters. Unsprayed control limbs were left on each tree and, where necessary were protected from spray drift with bituminous paper screens. Branches were sprayed to the run-off point. In the early experiments half of the branches sprayed at each stage of development received NAA only and half received NAA + 0·25% " Teepol " as a wetting agent. The wetting agent was found to have no influence on the effectiveness of the spray and was omitted in subsequent experiments.

Mode of action of NAA in reducing the crop.

Post-blossom applications of NAA reduce the ultimate set of fruit by increasing the extent of the " first drop " of developing fruitlets. Although it might seem paradoxical that a substance which is widely used for the prevention of pre-harvest drop of apples should be effective in promoting drop when applied at an earlier date, such a paradox does not in fact exist.

Recent work at Long Ashton on the physiology of fruit drop (4) has suggested that a continuous flow of natural hormone passing down the fruit stalk is necessary to prevent the abscission of the fruit, and that the bulk of this hormone normally comes from the endosperm of the developing seeds. When NAA is sprayed on the tree shortly after petal-fall it inhibits the growth of a proportion of the seeds, causing them to abort. This results in a reduced flow of natural hormone and, in consequence, an abnormally heavy drop of fruitlets.

There is usually a delay of about three weeks between the time of application of the thinning spray and the start of this induced drop. This is due to the well-known effect of NAA in delaying abscission, a property which is made use of for the control of pre-harvest drop. This delay in abscission is a direct effect of NAA on the separation of the cells at the base of the fruit stalk, and is not dependent on the presence of seeds. It must be emphasised, however, that this abscission-delaying effect of NAA is only temporary; after two to four weeks, depending on the concentration used and other factors, the effect wears off, and, in the absence of an adequate supply of natural hormone, the fruits will then start to drop.

It appears that the seeds are only susceptible to damage by NAA during the early stages of development, whilst the endosperm is still in the free-nuclear state. Once the endosperm has become cellular, applications of NAA have no effect on seed development, and hence no thinning effect. The precise time after petal-fall when thinning with NAA is no longer possible varies with variety and season. In 1950 it was found that Emneth Early could be thinned with NAA only up to two weeks after petal-fall; whereas with Crawley Beauty the spray was effective up to three weeks, and with Miller's Seedling up to four weeks from petal-fall.

TABLE I.

Effect of Thinning Three Varieties of Apples with Post-Blossom Sprays of Naphthalene Acetic Acid. 1950 Trials.

Variety	Conc. of NAA p.p.m.	Stage when sprayed	Fruits matured per 100 blossom clusters	Mean fruit weight (gm.)
Miller's Seedling	20	Control	61	39
		Petal-fall	*24*	*53*
		P.F. + 7 days	*27*	*51*
		P.F. + 25 days	*22*	*29*
		P.F. + 41 days	53	37
Emneth Early	20	Control	56	90
		Petal-fall	*26*	*63*
		P.F. + 14 days	44	50
Crawley Beauty	20	Control	63	72
		Petal-fall	*6*	99
		P.F. + 10 days	*15*	*90*
		P.F. + 20 days	*35*	*59*
		P.F. + 30 days	62	55
	40	Control	63	72
		Petal-fall	*3*	111
		P.F. + 10 days	*9*	70
		P.F. + 20 days	*26*	*55*
		P.F. + 30 days	63	*55*

Italic figures differ significantly from the control.

Concentration of NAA required.

In the 1950 trial NAA at a concentration of 20 p.p.m. thinned heavily cropping Miller's Seedling branches to about the right degree, reducing the number of fruits to between one-third and one-half of those on the control branches. The same concentration was satisfactory in reducing fruit numbers when applied at petal-fall to Emneth Early. Crawley Beauty, on the other hand, appeared more sensitive to NAA and 20 p.p.m. at petal-fall resulted in drastic overthinning. At ten days after petal-fall the same concentration

removed about three-quarters of the crop, which was approximately the degree of thinning required. At 20 days after petal-fall, on the other hand, 40 p.p.m. gave better thinning than did 20 p.p.m.

Experience with Laxton's Superb is limited to a single trial in 1951 when NAA at 30 p.p.m. was applied at petal-fall. This only reduced the final number of fruits per 100 blossom clusters from 63 to 39, which, in view of the heavy blossoming, gave barely adequate spacing of fruits along the branch.

It is clear from these preliminary trials that the concentration required will vary with the variety, the time of application of the spray and degree of thinning required, and that further experience with this method over a number of seasons will be required before it is possible to make any general recommendations regarding the best concentration to use under any given set of circumstances.

Effect on fruit size.

One of the objects of fruit thinning is to increase the proportion of fruit of marketable size. This is of particular importance in heavy cropping varieties such as Miller's Seedling and Crawley Beauty, where the unthinned fruits are usually too small, and in early cooking varieties such as Emneth Early, where the object is to obtain fruits of good size as early in the season as possible.

In Miller's Seedling and Crawley Beauty thinning with NAA 20 p.p.m. within ten days of petal-fall resulted in a very marked increase in fruit size (Table I). The petal-fall spray on Miller's Seedling, although it reduced the total crop weight by 45%, actually increased the proportion of dessert quality fruit (2″ or more in diameter) from 9·5% to 59·0%.

Where thinning was delayed beyond ten days after petal-fall, however, not only was this increase in fruit weight not obtained, but, in many cases, the fruits at harvest were considerably smaller than those from the unthinned control branches. In Emneth Early, where the experimental trees had set only a moderate drop, a marked reduction in fruit size resulted from spraying with NAA 20 p.p.m. as early as petal-fall.

It is clear from these experiments that NAA, in addition to its thinning action, has a retarding effect on fruit growth which, under certain circumstances, may offset any advantages gained by thinning. This retarding action is more marked the later the spray is applied. In order to overcome this disadvantage it is therefore necessary to apply the thinning spray not later than one week after petal-fall and to use a concentration not exceeding 20 p.p.m. In practice the thinning agent could probably be incorporated in the first post-blossom lime sulphur wash.

Trials with other growth-substances and inhibitors.

The chief disadvantage of NAA as a fruit thinning agent is undoubtedly its inhibiting effect on fruit development. The ideal post-blossom thinning agent would be one that inhibited the growth of the seeds, thus inducing increased fruit drop, without reducing the rate of growth of the remaining fruits. In 1951 a trial was carried out with a number of different substances to see if any of them fulfilled this requirement.

The substances tested included, in addition to NAA, two naphthoxy- and two phenoxy- growth substances, and three substances known to act as

growth-inhibitors or auxin antagonists. Each substance, in water solution, was applied shortly after petal-fall to two branches on each of four trees of Laxton's Superb. Two other branches on each tree were recorded as unsprayed controls. The trees were in their " on " year and had set a moderately heavy crop of fruitlets.

The results of this trial, given in Table II, show that, apart from NAA, the only substance which significantly reduced the number of fruits harvested was 2 : 4 : 5-trichlorophenoxy-acetic acid, and that this reduced fruit size to an even greater extent than did NAA itself.

TABLE II.

Effect of Petal-Fall Sprays of Various Growth-Substances and Inhibitors on the Cropping of Laxton's Superb. 1951 Trials.

Substance	Conc. p.p.m.	Fruits matured per 100 blossom clusters	Mean fruit weight (gm.)
Control—unsprayed	—	63	82
1–naphthalene acetic acid	30	*39*	*75*
2–naphthoxy–acetic acid*	75	66	89
α(2–naphthoxy–) propionic acid*	75	64	*75*
2:4:5–trichlorophenoxy–acetic acid*	50	*36*	*65*
α(2:4:5–trichlorophenoxy)– propionic acid* ..	50	65	*73*
Maleic hydrazide†	2000	51	*69*
Coumarin	1000	60	78
2:4 dichloroanisole*	100	64	81

Italic figures differ significantly from control.

* These materials were kindly supplied by Messrs. Pal Chemicals Ltd., London.

† Kindly supplied by Genatosan Ltd., Loughborough, Leicestershire.

A number of other substances reduced fruit size without exerting any significant thinning effect, viz. α–(2 naphthoxy) propionic acid, α(2 : 4 : 5–trichlorophenoxy)propionic acid, and maleic hydrazide.

In addition to its effect on fruit size it was noticed that α(2 : 4 : 5–trichlorophenoxy)propionic acid had a remarkable effect in stimulating the ripening of the fruit. When they were picked in the second week of September the fruits on these branches were obviously overripe and a large proportion of them had been destroyed by brown rot. The fruits which had not rotted were soft and had a bright orange ground colour, in marked contrast to the green ground colour of the unsprayed fruits. They also had abnormally thick stalks. It is interesting that 2 : 4 : 5–trichlorophenoxy acetic acid, which has previously been reported as active in advancing the maturity of apples (6) and pears (2), had no noticeable effect on the ripening of Laxton's Superb.

Effect on fruit-bud formation.

In addition to increasing fruit size in the " on " year thinning may be employed to encourage the formation of the fruit buds for the following year, thus promoting regular annual cropping in varieties which would otherwise bear biennially. In general it is found that the earlier such thinning is carried out, the more effective it is in influencing fruit bud formation.

There is little information available on the possible value of NAA for the control of biennial bearing. In the 1950 trials the branches of the strongly biennial Miller's Seedling which had been thinned with NAA, like the unthinned control branches, failed to form fruit buds. In the partially biennial Emneth Early and the annual cropping Crawley Beauty, the thinned branches carried approximately the same amount of blossom the following year as the controls. Although these observations suggest that NAA thinning is without effect on fruit bud formation, it would be unwise to draw firm conclusions from experiments based on branch treatments alone, since the thinned branches may have been influenced by the other unthinned branches on the same tree. However, it was observed that NAA applied to Miller's Seedling within three weeks of petal-fall retarded development of the spur foliage, and that during early June the thinned branches were carrying considerably less leaf area per spur than the unthinned controls. Since this is a critical period for fruit bud differentiation it is possible that this reduction in leaf area was largely responsible for the failure of the thinned branches to flower the following season.

The experiments of Fritzsche (3) at Wädenswil Research Station in Switzerland indicate that post-blossom thinning with NAA may be effective in controlling biennial bearing in some varieties but not in others. Trees of the varieties Sauergrauech, Champagner Reinette and Berner Rosen, which had been thinned with 50 p.p.m. NAA shortly after petal-fall all carried a moderately good blossom the following season, whereas the unthinned control trees failed to bloom. With Belle de Boskoop and Bohnapfel, on the other hand the same treatment was ineffective in bringing about fruit-bud formation.

Application to other fruit crops.

There appears at present to be no information available on the effect of post-blossom applications of NAA to plum trees. Should it prove to be an effective thinning agent it might provide a valuable means of preventing overbearing in certain varieties, thereby increasing fruit quality and reducing limb breakage.

Preliminary experiments with pears carried out at Long Ashton in 1950 indicate that the reactions of this fruit to post-blossom applications of NAA may be quite different from that of apples (Table III). Although the experiment was on a very limited scale, with only four treated and four control branches on each of two varieties, it is clear that no reduction in fruit set occurred following spraying with NAA at 20 p.p.m. On the contrary there seems to have been a general increase in crop on the sprayed branches, though, in view of the variability of the experimental material it would be unwise to attach too great a significance to these results. Not only did NAA fail to thin the crop, but it also had no marked inhibitory effect on fruit growth such as was observed with apples. Similar results have recently been obtained for the pear variety Beurré Alexandre Lucas by Vrijhof and Oele (7) working in Zeeland.

TABLE III.

Effect of Post-Blossom Sprays of NAA 20 p.p.m. on the Cropping of Two Varieties of Pear. 1950 Trials. The Treated Branches are Compared with Unsprayed Control Branches on the Same Trees.

Variety	Stage when sprayed	Fruits matured per 100 blossom clusters		Mean fruit weight (gm.)	
		Sprayed	Control	Sprayed	Control
Williams ..	Petal-fall	23	12	106	111
	P.F. + 7 days	36	28	102	97
Conference ..	Petal-fall	19	9	195	200
	P.F. + 7 days	19	21	167	193

Summary.

(1) Of the various types of thinning agents so far reported, naphthalene acetic acid (NAA) appears to be the most promising under English conditions because it can be applied as a post-blossom spray.

(2) When applied within two to four weeks of petal-fall NAA has been shown to induce seed abortion, thereby leading to an increased drop of young fruitlets.

(3) Preliminary trials with Miller's Seedling, Emneth Early, Crawley Beauty and Laxton's Superb are reported. The concentration required for adequate thinning depends on the variety, the time of application and other factors. Further experience is required before general recommendations can be made.

(4) One of the disadvantages of NAA as a fruit thinning agent is its retarding effect on fruit growth. This effect is less marked the sooner after petal-fall the spray is applied.

(5) Of seven other growth-substances tested only 2 : 4 : 5–trichlorophenoxy-acetic acid had any significant thinning action and this also reduced fruit size at harvest. 2 : 4 : 5–trichlorophenoxy-propionic acid caused the fruits of Laxton's Superb to ripen on the tree in August.

(6) Post-blossom applications of NAA at 20 p.p.m. to two varieties of pear had no thinning action.

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THE EFFECT OF MALEIC HYDRAZIDE IN DELAYING FLOWERING AND FRUITING.*

By HELEN M. FERRES.

Introduction.

Maleic hydrazide is a synthetic growth-inhibiting compound which has recently been reported as active in delaying the blossoming of certain fruits. White (1) found this effect particularly marked on raspberries (var. Bristol Black), the flowering of which was delayed for 24–38 days, and on Strawberry (var. Premier). No retardation of blossoming was obtained with apples (var. Golden Delicious). These experiments have been repeated at Long Ashton with other varieties of these fruits and the trials have been extended to include blackcurrants.

Raspberries.

Maleic hydrazide in water solution at concentrations of 750 and 1,500 p.p.m. was sprayed on canes of Norfolk Giant raspberry at "greenbud" stage (20/4/51). A second group of canes was sprayed three weeks later when about half the flower buds had opened. Control canes were not sprayed. Each treatment was applied to four replicate six-foot lengths of row, and the weight of fruit harvested from each length of row at successive pickings was recorded.

Spraying at greenbud had no noticeable effect on the time of flowering but there was a very marked reduction in the rate of development of the fruit and, with 1,500 p.p.m., a delay of the order of three to four weeks in the date of harvesting. There was no appreciable effect on the total weight of crop. Spraying during the blossoming period delayed ripening, but also caused malformation of the fruit and reduced the yield.

The failure to obtain a delay in blossoming in this experiment, such as was reported by White, may have been due to the later application of the spray. In White's experiment the canes were sprayed when the "leaflets were expanded to about 1 cm. and side branches were just beginning to appear," i.e. before the "greenbud" stage.

* This work was carried out at Long Ashton with the aid of an A.R.C. grant.

TABLE I.

Effect of Maleic Hydrazide on the Cropping of Norfolk Giant Raspberries. Crop Weights in Kgm. per 24 Feet of Row.

Stage when sprayed	Conc. p.p.m.	Date of picking							Total crop kgm.
		28·7	31·7	3·8	8·8	15·8	23·8	3·9	
Greenbud ..	0	3·2	0·8	0·5	0·3	0·1	0	0	4·9
	750	1·3	1·4	0·7	0·6	0·7	0·3	0·3	5·3
	1,500	0	0·3	0·2	0·6	1·1	1·0	1·0	4·2
Blossoming..	0	3·0	1·5	0·8	0·7	0·3	0·1	0	6·4
	750	0·4	0·5	0·1	0·6	1·0	0·9	0·5	4·0
	1,500	0	0	0	0·5	0·8	1·0	1·0	3·3

Strawberries.

Royal Sovereign strawberry plants were sprayed with maleic hydrazide at concentrations of 1,000 and 2,000 p.p.m. at four different stages of development, viz. : no flower buds visible ; buds just showing ; inflorescence developed ; first flower buds opening.

As in the previous experiment there was no delay in blossoming, but a marked retardation of fruit development and lengthening of the harvest period. At 2,000 p.p.m. there was considerable inhibition of vegetative development and reduction in total crop weight. These effects were most pronounced in the plants which were sprayed after the inflorescence had developed.

Blackcurrants.

Laboratory experiments in which blackcurrant buds were grown in sand watered with solutions of maleic hydrazide, showed that this compound was active in delaying bud opening at concentrations of 100–1,000 p.p.m. Similar results were obtained in experiments in which one-year-old shoots of blackcurrant were dipped in solutions of maleic hydrazide and then stood in jars of water in the glasshouse. In these experiments the delay in opening could be attributed partly to damage caused to the buds by the higher concentrations.

In the field no delay in flowering or fruiting of Baldwin blackcurrants was obtained by spraying with maleic hydrazide during the dormant period, at bud break nor at bud burst. The concentrations used were 10, 100, 750 and 1,000 p.p.m. The higher concentrations caused malformation of the leaves similar to that caused by "reversion," and, in addition, abscission of some of the berries.

Apples.

The application of maleic hydrazide to apples (Laxton's Superb) before and after bud break had no effect on subsequent development of the buds

or on the time of flowering. The concentrations used were the same as on blackcurrants.

Conclusions.

In these experiments no delay in flowering of raspberries, strawberries, blackcurrants or apples was obtained by the use of maleic hydrazide sprays at concentrations of the order of 10 -1,500 p.p.m. This compound was active, however, in retarding the development and ripening of the fruit in raspberries and strawberries.

In view of the report by Darlington and McLeish (2) that maleic hydrazide is a chromosome-breaking agent and that most of such agents are also carcinogenetic, the use of this compound for delaying the ripening of fruits would be ill-advised at present.

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INFLORESCENCE INITIATION AND ASPECTS OF THE GROWTH HABIT OF THE STRAWBERRY.

By C. G. GUTTRIDGE.*

The work reported here is part of an investigation into the growth habit of the strawberry, with reference to the influence of day length and low temperature on inflorescence and stolon formation. After a brief description of inflorescence development based on the work of Waldo (1) and others and confirmed by the present study, results of observations on the time of inflorescence initiation will be given, followed by a description of the growth habit as related to inflorescence formation.

Materials and Methods.

Certified plants of the varieties Royal Sovereign, Perle de Prague and Auchincruive Climax were planted in the field in April 1951, and, in addition, second year "Climax" plants (planted March 1950) were available. During summer the 1951 series of all three varieties were allowed to form stolon trains, and each young runner plant was labelled with the date of rooting. The stolons were not severed. At suitable intervals sample plants of each series were lifted, and after washing and removing leaves and roots, the crowns were fixed and preserved in formalin-acetic-alcohol, as this facilitated dissection. Dissections were done under a binocular microscope, × 16 and × 32 magnifications proving satisfactory for observing the growing points. Iodine in potassium iodide was used on occasions as a stain. Some paraffin sections were made. The illustrations are from camera lucida drawings.

*Postgraduate student of Reading University.

Formation of the Inflorescence.

The vegetative growing point is slightly domed in shape (Fig. 1), and at intervals of a week or longer, depending on the time of year, gives rise to leaf primordia in spiral succession. The first microscopical evidence of differentiation is a slight elongation of the growing point (Fig. 2). Following this the tip of the elongating growing point flattens out and sepal initials form round the top (Fig. 3). The flattened top later develops into the primary flower of the inflorescence. At this stage the secondary flowers will be visible as plain growing points placed laterally, in the axils of bracts, on the axis of the inflorescence (Fig. 3). Subsequently each individual flower develops sepal, petal, stamen and pistil primordia acropetally and in that order. The inflorescence is of the cymose type, although the actual structure varies considerably. The primary flower remains a week or more in advance of the secondary flowers, and similarly the secondaries are in advance of the tertiaries, and so on. The inflorescences arise terminally on both the main and the axillary axes.

The Time of Inflorescence Initiation.

Royal Sovereign. Inflorescence primordia were first seen on mother plants lifted on September 13th. The apices of all three plants lifted on this date had inflorescence primordia at the stage of development shown in Fig. 3. Plants lifted nine days earlier were vegetative. This suggests that differentiation started at about the end of the first week of September. Early-formed current-season runner plants differentiated on approximately the same date as their mother plants. Runner plants rooting after August initiated their first inflorescences soon after rooting.

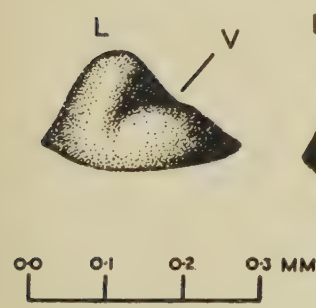

Fig. 1.

Fig. 2.

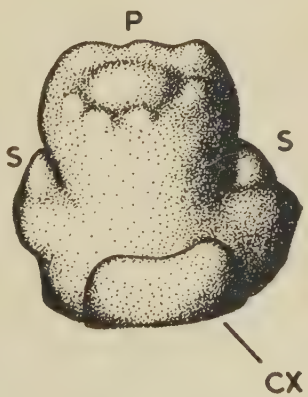

Fig. 3.

Fig. 1. Royal Sovereign. Vegetative growing point (V) with primordium of the last formed leaf (L).

Fig. 2. Royal Sovereign. Elongating growing point (D); the first sign of inflorescence initiation. Last leaf primordium (L).

Fig. 3. Auchincruive Climax. Sepal initials forming on the primary flower (P). Secondary flower initials (S). Crown extension bud (CX) in the axil of the uppermost primordial leaf (removed in dissection). Its first leaf is beginning to form on the right. Figs. 1–3 are on the same scale.

Perle de Prague. This variety commenced inflorescence initiation at about the same time as Royal Sovereign. The current season runner plants were not studied.

Auchincruive Climax. Inflorescence initiation occurred several weeks earlier in this variety. On July 6th a second year plant with six large crowns showed what appeared to be a differentiating apex on one of them. A plant lifted on July 24th had seven main crowns, and of these four were terminated by inflorescence primordia : two of them had sepal initials on the primary flower and the other two were less advanced. A second plant lifted on this date had five out of six crowns with terminal inflorescence initials, most of which were at or beyond the stage shown in Fig. 3.

Of five plants of the April 1951 series lifted on July 10th one showed an elongating growing point. On July 24th two plants each had inflorescence primordia terminating two small lateral buds, but the apices of the crowns were still vegetative. Later dissections showed that low laterals such as these were not infrequently carrying inflorescences more advanced than those terminating the main axes of the plants. This phenomenon occurred but rarely in the 1950 series and was not seen in the other varieties. On August 10th four plants were lifted ; one of them had both of its main crowns terminated by an inflorescence whose primary flower was developing pistils—the same stage of development as plants of the 1950 series on this date—two of them had inflorescences at the same stage on laterals and the fourth was still vegetative. It is concluded that microscopic inflorescence primordia were present in " Climax " from about the second week of July, although there was more variation in the time of differentiation in this variety than in Royal Sovereign.

Inflorescence initiation in current season runner plants of this variety started at a later date than in their mother plants, first signs of differentiation occurring in August. This confirms the unpublished observations of R. D. Reid (private communication). As with Royal Sovereign, the later-formed runner plants commenced differentiation later in the season. The difference between runner and mother plants remained throughout the autumn, so that, whereas the mother plants came into flower in September or October, the runner plants carried flowers only exceptionally in the autumn and even then only very late. The autumn flowering habit of Auchincruive Climax is thus accounted for by the early commencement of flower initiation compared with the other two varieties studied.

Inflorescence Formation and the Growth Habit.

The preceding remarks on the time of inflorescence initiation have referred only to the first formed inflorescence, arising terminally on the crown. Differentiation, however, continues throughout the autumn until growth ceases or almost ceases in winter, and several inflorescences may be formed on a single crown, as will be described.

The growth habit is sympodial. Following differentiation of the primary growing point into an inflorescence there is a loss of apical dominance, and vegetative growth continues from the bud in the axil of the leaf primordium immediately below the differentiated primary growing point. At the same time the bud primordia in the axils of lower

leaves on the primary* axis will also grow, and normally most of them will develop terminal inflorescences, after the formation of a varying number of leaf primordia, typically four. Some of these lower laterals may subsequently develop into branch crowns. They will be referred to again below. Meanwhile extension growth of the crown will be continued by the vegetative growth of the uppermost axillary axis. This axis will be referred to subsequently as the secondary crown-extension axis. After forming two to four leaf primordia (or more) this crown-extension axis will, in turn, develop a terminal second order inflorescence, and vegetative growth will continue from the axillary bud immediately below it, to form a tertiary crown-extension axis. This habit of growth continues through the autumn until winter. It is probable that these varieties do not initiate further inflorescences in the spring. By December a large crown normally consists of a primary axis terminated by a first order inflorescence, a secondary crown-extension axis terminated by a second order inflorescence, and a tertiary axis which may also be terminated by an inflorescence—a third order inflorescence (Fig. 4). Crown extension growth the following year continues from the axillary bud immediately below the highest order inflorescence. Casual observation does not reveal the sympodial structure of the crown.

Growth of the lower axillary buds on the main axis continues more or less slowly, and many of them develop terminal inflorescences, as already mentioned. When growing conditions are favourable for crown multiplication some of the lower laterals make vigorous growth and develop as small branch crowns in the autumn, and their inflorescences develop in the normal manner and come into flower the following summer. When conditions are less favourable for branch crown formation most of these laterals develop very slowly, and remain, during the autumn, as buds hidden in the axils of their subtending leaves. Frequently the second axillary buds below both the first and second order inflorescences grow out as branch crowns the following summer, soon after the inflorescences emerge from the crowns (Reid, private communication). Any inflorescences present in these buds normally flower at the same time as those on the crown-extension axes. Of the lower lateral buds that grow only slowly in the autumn some may grow out as branch crowns at a later date, others die, and yet others may start to grow out the following summer, throw a weak inflorescence, and then die. The formation of branch crowns is beyond the scope of this paper and further comment will not be made.

Typical structure in late autumn.

In December 1951 the Royal Sovereign mother plants usually carried first and second order inflorescences on each crown, although some of the large crowns had a third order inflorescence, typically at the stage of development shown in Fig. 3. The first order inflorescences were in an advanced stage of development and almost ready to emerge from the crowns. The second order ones on the secondary crown-extension axes, being differentiated later, were less advanced. Where lower buds were developing as branch crowns their inflorescences were at varying stages of development according to the position of the buds on the crown.

*The designation of axes as primary, secondary, etc., is arbitrary, as each axis has developed as a lateral of some other earlier formed axis, and so on back to the original seedling of the clone. The terms first order, second order, etc., are applied in this paper only to the inflorescences borne on the primary and crown-extension axes.

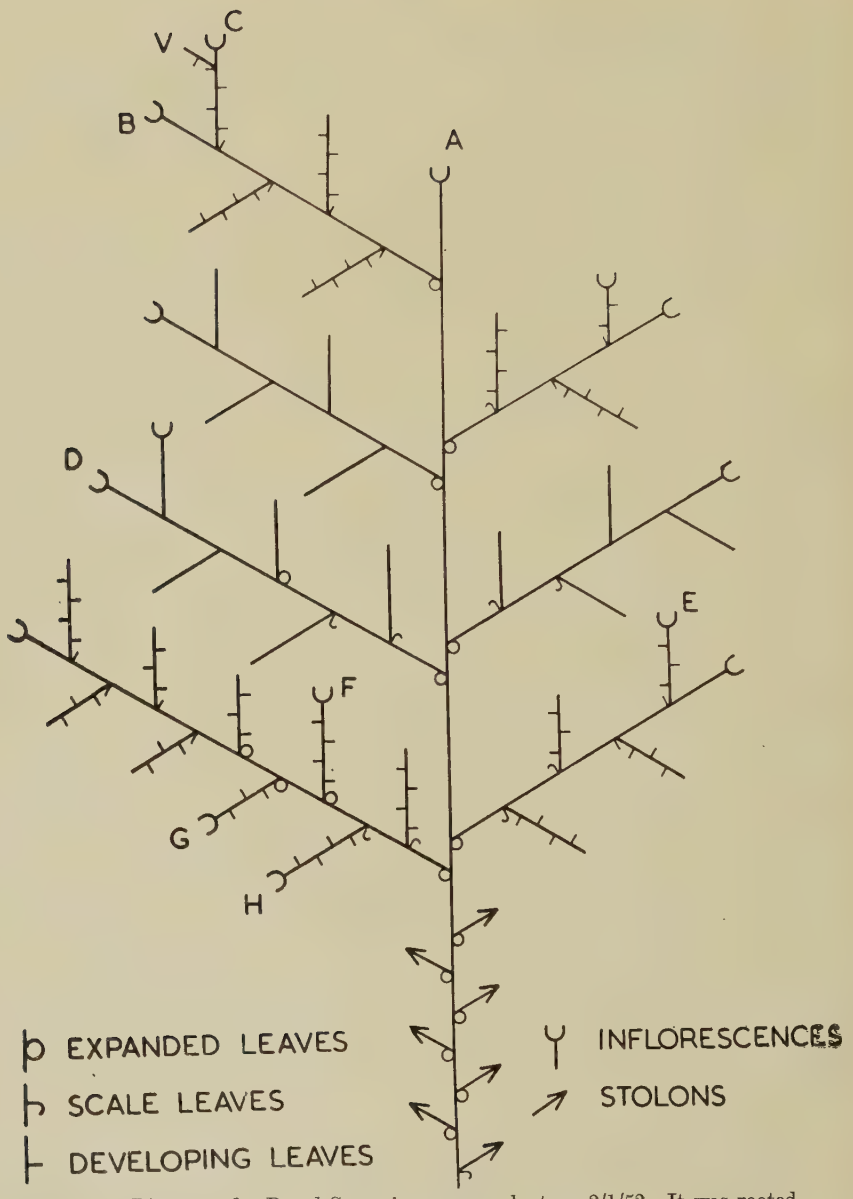

Fig. 4. Diagram of a Royal Sovereign runner plant on 2/1/52. It was rooted on 17/7/51.

A.—Inflorescence terminating the primary axis, and about to emerge from the crown. B.—Advanced inflorescence (primary flower developing pistils) terminating the secondary crown-extension axis. C.—Inflorescence at stage shown in *Fig.* 3 terminating the tertiary crown-extension axis. The inflorescences (e.g. D) terminating all the main lateral axes were at approximately similar stages of development to inflorescence B. Inflorescences E, F, G, H were at or near the stage shown in *Fig.* 3. The lowest lateral had developed as a small branch crown and had already expanded three true leaves. Vegetative growth of the main crowns would have continued next season from axis V. The developing leaves are shown on some axes only. "Developing leaves" includes all primordial and developing leaves enclosed within the stipules of the last expanded leaf.

The older current season runner plants of Royal Sovereign carried first, second and occasionally third order inflorescences in a similar way to individual crowns on their mother plants. The last formed runner plants had only first order inflorescences. A diagramatic representation of a Royal Sovereign runner plant (rooted 17 7 51, lifted 2/1/52) is shown in Fig. 4. Crown multiplication is occurring. The larger individual crowns of mother plants are essentially the same when crown proliferation is taking place.

Following the earlier flower initiation in Auchincruive Climax mother plants the inflorescences of this variety were more advanced throughout the autumn than those of Royal Sovereign. The first order inflorescences, initiated in July, emerged from the crowns towards the end of August or early in September, and subsequently flowered in September or October. Owing to frosts fruits were rarely formed. By December the second order inflorescences were almost ready to emerge from the crowns and were therefore at about the same stage of development as the first order inflorescences on Royal Sovereign. Third order inflorescences were found more frequently in this variety than in Royal Sovereign, as would be expected from the earlier commencement of differentiation. These were similar to, or perhaps slightly less advanced than second order Royal Sovereign inflorescences.

Current season Auchincruive Climax runner plants were less advanced by December than their mother plants, which is consistent with their later inflorescence initiation. Typically, the earlier formed runner plants were showing first order inflorescences emerging from the crowns towards the end of November. Only rarely did flowers open in the autumn and the primary flower stalks did not elongate.

Royal Sovereign and Auchincruive Climax appear to be similar in general in the development of inflorescences on their lower laterals, although, as mentioned already, certain "Climax" plants of the 1951 series initiated inflorescences on some of the lower laterals before doing so on the primary axes. These inflorescences were apparently initiated in July on lateral buds formed the previous autumn, although the possibility remains that they were autumn initiated and delayed in their development. This point requires further investigation.

After remaining dormant in winter the plants come into growth in spring and the inflorescences and leaves emerge in the order of their arrangement on the crown. Although no crop weights have been recorded, preliminary observation suggests that the major portion of the crop is borne on : (1) The inflorescences terminating the primary and crown extension axes ; (2) The inflorescences arising on those laterals which develop as branch crowns. Inflorescences on laterals other than those developing as branch crowns in autumn or spring appear to be of little importance.

It is emphasised that the observations recorded in this paper were made on plants grown for one season only. Moreover, the range of growing conditions was narrow and in only one variety were two year old plants studied. The 1951 series were unavoidably planted late. Further observations will be made in 1952, and this report must be considered as preliminary. The data on the time of inflorescence initiation were collected from small samples, but the results are to some extent confirmed by the consistent development of the inflorescences observed in dissections made during the autumn.

Acknowledgments.

Most of the work reported here has been carried out at Long Ashton Research Station and the author wishes to express grateful thanks to the Director for making this possible.

The author is greatly indebted to Professor R. H. Stoughton, University of Reading, and to Dr. L. C. Luckwill, Long Ashton, for their supervision and constant help throughout the year, and to Mr. R. D. Reid † at Auchincruive, for many suggestions and for generously allowing access to his unpublished observations on the growth habit of Auchincruive Climax. Mr. Reid also kindly supplied some of the plants.

Grateful thanks are expressed to numerous members of the staffs at Long Ashton and Reading University for their assistance.

The Ministry of Agriculture post-graduate scholarship received is gratefully acknowledged.

Summary.

(1) It is concluded that the first microscopic changes in the growing point indicating inflorescence initiation were present in Royal Sovereign plants at the end of the first week of September.

(2) Perle de Prague commenced inflorescence initiation at about the same time.

(3) Auchincruive Climax initiated inflorescences from the second week of July. The time of differentiation varied more widely in plants of this variety than in Royal Sovereign. The first formed inflorescences gave rise to the autumn flowers of " Climax " mother plants.

(4) The sympodial habit of growth and certain aspects of branch crown formation are described with reference to inflorescence formation.

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† Of the Scottish Horticultural Research Institute, stationed at the West of Scotland Agricultural College, Auchincruive.

NUTRIENT PLACEMENT IN RELATION TO FRUIT TREE NUTRITION.

III. NITROGEN FERTILIZATION OF APPLE WITH FOLIAGE SPRAYS OF UREA. PROGRESS REPORT.

By C. BOULD and J. TOLHURST.

In 1943 Hamilton *et al* (5) published the first account on the use of Uramon (urea) as a means of regulating the nitrogen supply of apple trees. They found that urea, at the rate of 5 lbs. per 100 gals., could be mixed with lime sulphur and lead arsenate sprays, without causing any damage to the leaves. Furthermore, by using urea sprays they could control the nitrogen status of the leaves more easily than by soil application of nitrogen fertilizers. They suggested that such a method might prove particularly useful during periods of low rainfall and in dry areas.

Fisher *et al* (3, 4) compared foliage sprays of urea with soil applications. They found that time of application of sprays was most important. Early foliage sprays increased set and colour of fruit, whereas mid-summer sprays tended to increase size of fruit and reduce colour. Boynton (1) also stressed the importance of time of application of urea sprays. He states that when the first foliage spray was given at petal-fall, set, growth and yield were no better than on the unfertilized trees, and colour was inferior. When the first application was made in the late pink-bud stage, and the last application at the time of the first cover spray, the set and yield of fruit were higher than for soil applications and the colour of fruit was good. In a later paper Boynton (2) states that 1 lb. of nitrogen per tree as urea in early post-blossom sprays has about the same effect on yield and quality of fruit as 1 lb. of nitrogen broadcast under the branches in early spring. He says that substitution of urea sprays for soil application will not solve all the nitrogen problems but may help if used to supplement good soil management and fertilizer practice.

In 1950 an experiment was started by us to study the effect of foliage sprays of urea on the nitrogen status of apple leaves, a preliminary account of which follows.

Experimental.

The experiment is being carried out in a commercial orchard of Laxton's Superb bush apple trees on Malling No. I rootstock. The trees were planted in 1941 at 18′ × 18′ and at present are growing in a grass plus clover sward which is kept short by frequent gang-mowing.

Layout and Treatments.

An area, comprising 24 trees, was marked out in the spring of 1950 for spraying purposes, the whole area being enclosed by a single row of guard trees. No manures have been applied to this experimental area since the spring of 1949.

The area was divided into six blocks, each of four trees, each tree receiving a different treatment. The treatments are :—

1. Control—no spray.
2. One spray of 1% urea (10 lbs. per 100 gals. water).
3. Two sprays ,, ,, ,, ,, ,, ,, ,, ,, ,,
4. Three ,, ,, ,, ,, ,, ,, ,, ,, ,, ,,

Sufficient Estol "H" wetting agent was added to the urea sprays to give a continuous film on the leaves. No attempt was made to control the volume of spray applied to each tree. The dates of application and average amounts of spray per tree were :—

	1950.			1951.	
	Date.	*Volume per tree.*		*Date.*	*Volume per tree.*
1st spray	June 2	1·7 gals.	..	May 31	1·4 gals.
2nd ,,	June 12	2·0 ,,	..	June 22	1·7 ,,
3rd ,,	July 12	2·0 ,,	..	July 6	1·7 ,,

No leaf damage was caused by the urea sprays in these years.

Results.

Results 1950.

No visual differences in foliage or fruit colour between treatments could be seen at any time during the season. Although the trees appeared to be low in nitrogen prior to the treatments, the general nitrogen status improved throughout the season and in September the nitrogen level in the leaves of control trees was too high for good quality fruit. This might be due to the high clover content of the sward and the rapid turn-over of nitrogen caused by the gang-mowing.

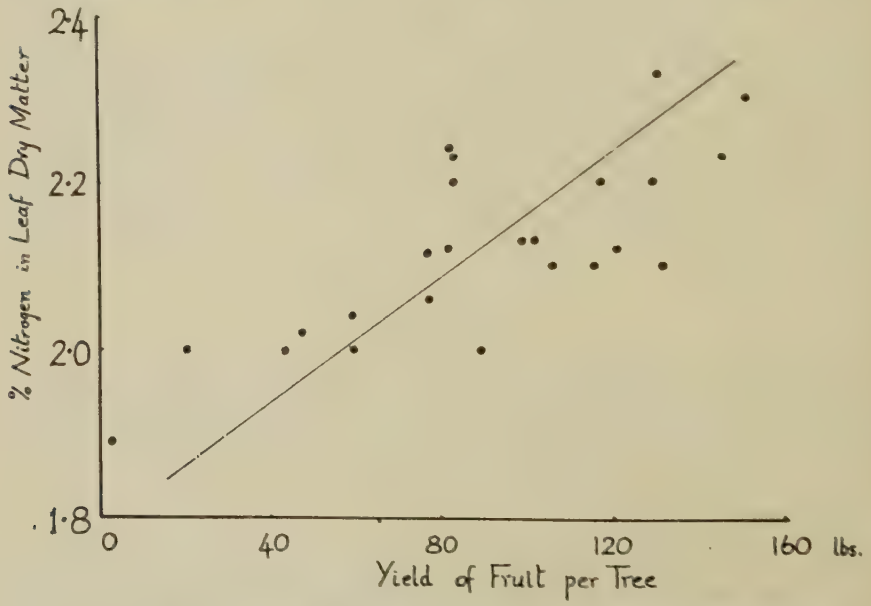

Fig. 1. Showing the relationship on a tree basis between leaf nitrogen as a percentage of dry matter and yield of fruit in lbs. Leaves sampled 22.9.50. Correlation coefficient $r = + 0{\cdot}800$.

Leaf samples were taken on 22 9 50 and analysed for total nitrogen.

The treatments themselves had no significant effect on leaf nitrogen content or yield of fruit. However, when leaf nitrogen content was plotted against yield of fruit on a *tree* basis a significant positive correlation was shown (correlation coefficient r = 0·800). This relationship is illustrated in Fig. 1. The regression coefficient of yield on leaf nitrogen = 277. This means that for an increase in leaf nitrogen of 0·1% over the range given in Fig. 1. the yield was increased by 27·7 lbs. per tree. Unfortunately at this high nitrogen level quality of fruit, as measured by size and colour, is poor. Magness (6) has shown a negative correlation between leaf nitrogen content and colour of fruit. He found that a reduction of 0·1% in the leaf nitrogen (as a % of dry matter) resulted in an increase in fruit colour of about 5%.

Results 1951.

No visual differences due to treatments could be seen in leaf colour, extension growth or fruit quality. The fruit quality was marred by the development of late scab in spite of frequent fungicidal sprays. No yield data were available.

Leaf samples were taken on three separate occasions, since other workers have shown that differences in leaf nitrogen due to treatment may diminish as the season progresses. The analytical results are given in Table I. together with tests of significance, and are illustrated in Fig. 2. The low mean value for nitrogen at the first sampling from the trees receiving one spray (treatment 2) was due to a single anomalous tree.

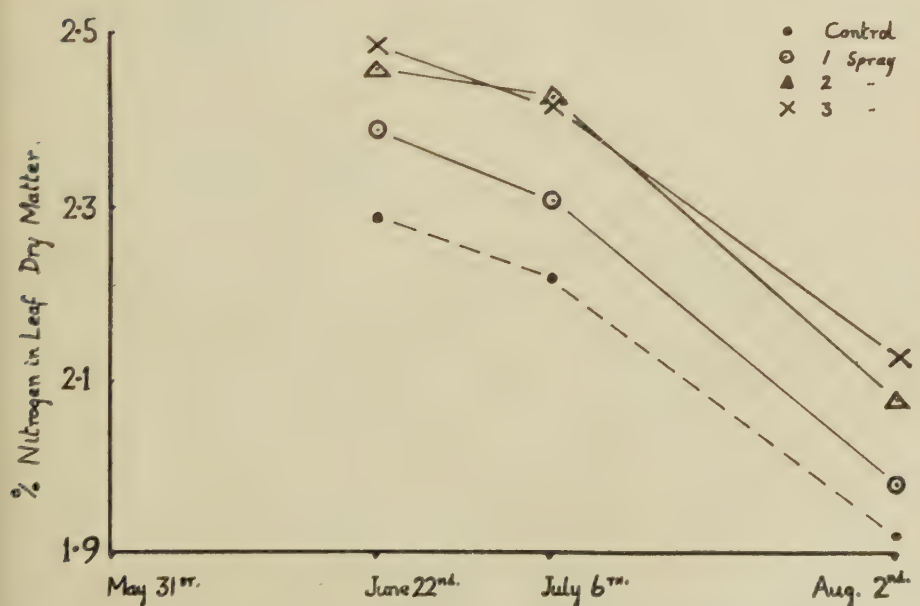

Fig. 2. The effect of foliage sprays of 1% urea on the nitrogen content of apple leaves. First spray applied May 31st, second spray June 22nd, third spray July 6th.

TABLE I.

EFFECT OF UREA FOLIAGE SPRAYS ON THE NITROGEN CONTENT OF LEAVES: VAR. LAXTON'S SUPERB, 1951

Sampling date	Mean total nitrogen as % leaf dry matter				S.E. Mean	L.S.D. $P = \cdot 05$	L.S.D. $P = \cdot 01$	Remarks
	T_1—Control	*T_2	*T_3	*T_4				
22/6/51	2·293	2·398	2·460	2·497	0·0431	0·130	N.S.	$P = \cdot 05$ T_4 $T_3 > T_1$.
6/7/51	2·220	2·306	2·430	2·420	0·0294	0·089	0·123	$P = \cdot 01$ T_4 $T_3 > T_2$ T_1.
2/8/51	1·925	1·982	2·078	2·133	0·0361	0·109	0·150	$P = \cdot 01$ $T_4 > T_2$ T_1. $T_3 > T_1$.

L.S.D. = Least difference between means for significance.
* T_2 = Trees sprayed May 31st.
T_3 = Trees sprayed May 31st and June 22nd.
T_4 = Trees sprayed May 31st, June 22nd and July 6th.

It is clear from these results that foliage sprays increased the leaf nitrogen content. The first spray, i.e. at petal fall, had the greatest effect, the second spray had less effect but it was still significant and the third spray had a small but non-significant effect on the nitrogen status of the leaf. It will be noticed too that the effect of the first spray diminished as the season progressed.

It is hoped to continue these experiments in order to study residual effects of foliage sprays and the relationship between treatments, nitrogen status of the leaf and fruit quality.

Acknowledgments.

We should like to express our thanks to Mr. E. W. Pinks, Mazzard Fruit Farm, Ottery-St.-Mary, for granting facilities for this experiment and for his help and co-operation, and to Miss P. Scadding for help with the analytical work.

Summary.

(1) An account is given of an experiment to determine the effect of foliage sprays of urea on the nitrogen status of apple leaves.

(2) Urea sprays at 1% concentration (10 lbs. per 100 gals.) caused no damage to apple leaves when applied at petal-fall and later.

(3) Treatments had no effect on leaf nitrogen and yield of fruit during the first season. This was due to the general high level of nitrogen in all the trees.

(4) During the second year treatments had a significant effect on the leaf nitrogen status. The greatest effect was produced by the petal-fall spray, later sprays having less effect.

(5) A significant positive correlation was shown to exist between leaf nitrogen status and yield of fruit in the first season.

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MOLYBDENUM AS A PLANT NUTRIENT. III. THE EFFECTS OF MOLYBDENUM DEFICIENCY ON POTATO, CARROT, RADISH AND SOME OTHER CROPS.

By E. J. HEWITT.

This paper reports preliminary results obtained in attempts to induce molybdenum deficiency in the potato in sand culture, and yields and the visual symptoms of molybdenum deficiency observed in seven crops grown to test the relative suitability of glass-distilled and demineralised rainwater supplies.

Experimental.

Methods.

Potato tubers (Majestic) weighing between 30 and 60 g. and already sprouted were set in purified sand cultures in July, 1950. The usual methods to eliminate molybdenum (5) were used. After 10 days the rooted shoots were lifted and the tubers removed from the rooted sprouts which were re-planted. Normal haulms developed and tubers were saved for re-planting in April, 1951. For this purpose three tubers (20–60 g.) from molybdenum-deficient and two from control cultures (30–50 g.) were selected.

For the test of water supplies to be used in large scale work seven crops were used : swede, lettuce, radish, carrot, tomato, red clover, and sugar beet. Most were indicator crops of known reaction. Carrot and radish were included to obtain new records.

The plants were grown in 1·5 L. tall pyrex beakers each with a central drainage hole. These were cleaned by detergent, acid and steaming after previous use at normal or deficiency levels of molybdenum. Sand was purified as described earlier (5). The available molybdenum (3) was 0·00005–·0001 p.p.m., and was thus appreciably higher than in previous work. Nutrient reagents were treated with copper and hydrogen sulphide (5).

The water supplies tested were : (a) glass-distilled water obtained by a single distillation, from continuous operating pyrex glass stills, of softened tap water distilled first from tinned copper stills with silver condensers ; and (b) demineralised rain water. This was passed first through five Permutit ion-exchange resins (1) and finally through 15 L. Industrieele Activit IMAC C 11 cation-exchanging resin held in a pyrex glass cylinder 27 in. long by 6 in. diameter. This resin had been previously tested (3) and shown under laboratory conditions to remove up to 99·5% of molybdenum initially present as molybdate at microgram concentration levels.

The two water supplies were used to prepare and dilute the nutrient solutions which were applied daily (or initially 2–3 times weekly). There were two blocks for each type of water supply.

Visual symptoms and growth.

Potato. No visual symptoms developed in 1950 but yields were apparently reduced (Table I). In 1951 visual effects were observed in the deficient plants. They were consistent for each pot, and were entirely absent from the control plants given molybdenum.

TABLE I
YIELD (G. DRY WT. PER PLANT) OF POTATOES.

Part	Without Molybdenum				With Molybdenum			Mean ratio %
				Mean			Mean	
Haulm and root —1950 ..				24·0			39·0	61·5
Tuber—1950 ..				81·35			138·2	58·7
Total				105·35			177·2	59·5
	Pot 1	Pot 2	Pot 3		Pot 1	Pot 2		
Haulm—1951..	42·01	52·55	48·78	47·78	82·28	78·04	80·16	59·6
Root—1951 ..	10·12	13·89	11·74	11·92	20·0	10·28	15·14	78·5
Tuber—1951								
(Fresh) ..	(748)	(686)	(555)	(663·0)	(961)	(914)	(937·5)	(70·8)
(Dry) ..	157·1	143·8	116·3	139·1	200·0	191·0	195·5	71·1
Total	209·2	210·2	176·8	198·80	302·3	279·3	290·80	68·25
1951 Seed tuber at planting (g.)	54 (Fresh wt.)	45	24	41	56 (Fresh wt.)	26	41·0	—
Seed tuber at harvest (g.)	59	36	20	38	48	32	40·0	—

	−Mo	+Mo
% Dry wt. in tubers :	1950, 23·3 ; 1951, 21·0	1950, 20·0 ; 1951, 19·7.

The first symptoms appeared early in July about 12 weeks after planting. Rapidly expanding or recently fully expanded leaves were mainly concerned. Foliage became pale, followed by an unusual golden chlorosis and sometimes a dull white mottle. Leaf margins curled upward, were often darker than central areas and then showed irregular necrosis and scorching followed by total withering of the leaflets. The youngest leaflets and petioles on unexpanded leaves became brown and withered and death of the growing point and unopened flowers occurred. Lateral shoots developed and repeated the cycle of events. The typical appearance is shown in Plate I*a*. Yields were reduced (Table I) but to a slightly lesser extent than in 1950 when the tubers were removed.

There was nothing to suggest that the visual effects were due to virus infection and they did not resemble other known deficiencies, except possibly certain aspects of boron and phosphorus deficiencies or "acidity" effects reproduced by high potassium and excess of iron (7).

Radish, carrot and other crops. The behaviour of tomato, red clover, lettuce, sugar beet and swede confirmed in general previous observations (4, 5, 6). Symptoms in red clover were more acute than recorded in 1949.

In the other crops severity was rather less than recorded previously. This was consistent with the difference in residual available molybdenum in the sand. Lettuce plants were usually killed at an early stage but some survived. In these the leaves were narrow with scorched margins, resembling specimens from a molybdenum deficient site in Wiltshire (8). Sugar beet plants developed intervenal chlorosis and marginal mottling of middle leaves before scorching. Swede plants recovered from initially severe symptoms and later developed Whiptail symptoms. These originated by breakdown of leaf margins which became brown and deliquescent before expansion. (Plate I*b*).

Radish showed pronounced yellow-green or yellow intervenal chlorosis followed by marginal cupping, scorching and withering of older and middle leaves (Plate I*c*). Contrary to other Cruciferae—Brassica crops studied previously—, there was no indication of secondary symptoms leading to Whiptail after partial recovery. Carrot foliage became pale green, yellow-green and scorched along the segments. Leaves finally withered and collapsed. Symptoms were not diagnostic and would be readily confused with other mineral deficiencies.

Effect of Water Supply.

Typical visual effects and reduction in growth were seen irrespective of water supply. Initially their severity was greater or symptoms progressed more rapidly where glass-distilled water was used, but the difference was not usually striking. Tomato, swede and radish showed the greatest differences in the early stages. Later there was apparently some slight contamination of the glass-distilled water and the plants given demineralised water developed the most severe effects (Plate I*d*). Yield data for early and final harvesting reflect this change (Table II).

The demineralised water was tested twice by the *A. niger* technique (3) during the experiment which lasted four months. There was apparently 0·00001–0·000015 p.p.m. Mo present compared with 0·000005–0·000008 p.p.m. Mo in glass-distilled water. The molybdenum in demineralised water was obviously low enough for many purposes including the production of visual symptoms, growing plants for biochemical investigations, and was well below the critical level of 0·00005 p.p.m. at which typical field symptoms of Whiptail appear in cauliflower (2).

Conclusions.

(1) Potato plants may require at least two years' growth under deficiency conditions before showing a visual response to molybdenum deficiency.

(2) Swede plants may develop Whiptail effects practically identical with cauliflower when grown at low but still appreciable levels of molybdenum, whereas radish, also a Crucifer, may not behave similarly at the concentrations present in sand and water.

(3) Demineralised rain water is satisfactory for work on many aspects of molybdenum nutrition and will permit acute deficiency symptoms to develop. The possible Mo content does not greatly exceed that in glass-distilled water.

a. Effects of molybdenum deficiency in potato. *Left* : Shoot from control plant. *Right* : Shoot from Mo deficient plant showing chlorosis and curling of leaf margins. *Bottom centre* : Advanced stages showing necrosis of the growing point.

b. Swede plant showing Whiptail habit induced by molybdenum deficiency.

c. Molybdenum deficiency in radish, showing chlorosis, leaf cupping and withering. Note that the cotyledons are still normal.

d. Effects of water supplies on production of molybdenum deficiency in red clover. *Left* : Demineralised rainwater. Right : Glass distilled water.

PLATE I.

TABLE II.

YIELDS (DRY WT. G. PER PLANT) OF CROPS GROWN IN TEST OF WATER SUPPLIES.

Crop	Harvest	Glass-distilled Water			Demineralised Water		
		I	II	Mean	I	II	Mean
Tomato ..	18/7/51	1·77	1·49	1·63	3·95	6·89	5·42
	3/9/51	14·22	18·88	16·55	20·70	21·96	21·33
Sugar beet ..	18/7/51	0·64	0·17	0·41	1·36	0·92	1·14
	21/8/51	2·96	4·98	3·97	13·76	1·36	7·56
Swede ..	18/7/51	2·57	1·30	1·94	3·62	2·35	2·99
	3/9/51	23·86	22·93	23·40	22·69	25·81	24·25
Radish ..	18/7/51	1·40	2·07	1·74	5·83	6·08	5·96
	3/9/51	21·49	Dead	21·49	25·27	Dead	25·27
Red Clover ..	3/9/51	2·23	5·84	4·04	4·73	1·45	3·09
Carrot ..	3/9/51	0·91	4·24	2·58	1·36	1·28	1·32
Lettuce ..	21/8/51	0·28	Dead	0·28	0·15	Dead	0·15

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THE EFFECTS OF ZINC AND COPPER DEFICIENCIES ON CROP PLANTS GROWN IN SAND CULTURE.

By E. J. HEWITT and E. W. BOLLE-JONES.

The work reported here comprised : (a) a preliminary survey of the comparative effects of zinc and copper deficiencies on large and small seeded legumes, (b) a trial to find how the duration of sand purification treatments with acid, and the source of water, affected the incidence of these deficiencies in tomato and red clover as test crops.

Experimental.

Methods.

The methods used were similar to those already outlined by Hewitt and Jones (1951). Sand was extracted either twice or three times for 8 hour periods with acid as described before. 1 gallon urns or 5L pyrex glass beakers were used to hold the sand. The water supplies were as follows :—

(i) Water distilled from electric stills with tinned copper boiling pans and pyrex glass condensers. (ii) Water from stills with tinned copper pans and solid silver condensers, subsequently re-distilled once from pyrex glass stills ; (iii) Similar water re-distilled twice from pyrex glass stills : (iv) Rain water first demineralised, as described by Hewitt (1947) but using 5 Permutit units, and then passed through a pyrex glass cylinder $5'' \times 27''$ containing IMAC C11 cation resin at 4 L per hour. For the general survey the once-glass-distilled water supply (ii) was used.

The crops grown were pea (Onward), dwarf bean (Masterpiece), runner bean (Princeps), broad bean (Triple White), alsike clover (*T. hybridum*), red clover (Dorset Marl), Lucerne (Provence) and tomato (Market King).

Seeds were sown direct in the sand and thinned later. Cotyledons of dwarf bean and broad bean were excised immediately after emergence in one pot in each treatment to test the possible effects of seed reserves.

There were two replicates in each treatment arranged in two blocks.

Results.

Survey of legumes. The large seeded species (beans and pea) were outstandingly more resistant to copper deficiencies than the small seeded ones, but were slightly more susceptible to zinc deficiency when judged on a yield basis, although visually there were no marked differences. Lucerne was severely affected by copper deficiency and less so by lack of zinc. Alsike and red clover were slightly less sensitive than lucerne to copper deficiency and also to zinc, the effects of which were generally markedly less acute than those of copper.

Removal of the cotyledons of dwarf bean clearly accelerated the first symptoms of copper deficiency and reduced yields in both deficiencies. The area of prophylls (judged visually) was obviously reduced by the removal of cotyledons in all treatments, including complete nutrient, but the effect on growth in the latter did not persist visually for long. No abnormal effects were seen, but yields in the complete nutrient treatment were slightly

TABLE I.

EFFECTS OF COPPER AND ZINC DEFICIENCIES ON GROWTH OF PLANTS IN SAND CULTURE.

Crop, etc.		Copper deficiency			Zinc deficiency			Complete Nutrient	
		Mean total Fresh wt.	Dry wt.	% of Complete Dry wt.	Mean total Fresh wt.	Dry wt.	% of Complete Dry wt.	Fresh wt.	Dry wt.
Dwarf bean	Cotyledons attached	77·36	10·78	18·55	94·50	16·31	28·10	280·5	58·20
" "	Cotyledons removed	53·85	6·96	12·55	87·50	12·20	21·84	262·5	55·45
Runner bean		113·42	19·04	19·50	119·74	19·50	19·96	738·1	97·53
Broad bean	Cotyledons attached	77·19	12·07	22·28	56·38	7·58	14·03	394·2	54·14
" "	Cotyledons removed	5·27	1·53	3·81	25·00	5·46	13·63	302·3	40·05
Pea		56·42	7·31	36·86	37·75	4·31	21·73	113·5	19·89
Alsike clover		1·35	0·19	1·60	34·00	5·00	42·00	172·05	11·87
Lucerne		1·51	0·15	1·44	38·85	5·58	53·72	88·50	10·38
Red clover	Sand treatment								
Tinned copper stills	2 days	48·41	6·50	56·21	46·00	8·38	72·42	—	—
" " "	3 days	57·74	7·95	69·70	61·20	8·75	75·60	—	—
Demineralised rain water	2 days	3·90	0·46	3·87	21·00	2·80	24·18	100·4	11·58
" " "	3 days	5·50	0·68	5·88	25·33	3·06	26·44	—	—
Once glass distilled	2 days	1·35	0·205	1·73	29·75	4·31*	37·24	—	—
" " "	3 days	1·98	0·32	2·61	15·38	1·80	15·55	—	—
Twice glass distilled	2 days	1·64	0·155	1·34	42·00	4·80*	41·50	—	—
" " "	3 days	2·01	0·20	1·73	91·60	7·50*	64·77	—	—
Tomato	Sand treatment								
Tinned copper stills	2 days	105·4	14·86	24·24	161·2	21·71	35·58	—	—
" " "	3 days	112·3	13·48	22·20	130·3	24·15	40·00	—	—
Demineralised rain water	2 days	36·05	3·29	5·42	93·87	16·71	27·56	802·8	60·59
" " "	3 days	13·84	1·17	1·93	100·8	17·04	28·05	—	—
Once glass distilled	2 days	23·97	2·32	3·82	59·11	14·78	24·18	—	—
" " "	3 days	17·58	1·61	2·66	66·38	14·54	23·97	—	—
Twice glass distilled	2 days	21·11	1·92	3·16	113·4	13·98	23·04	—	—
" " "	3 days	12·57	1·37	2·26	104·4	20·55*	33·80	—	—

* Possibly contaminated by precipitation or paint from roof.

depressed. Removal of cotyledons of broad bean seedlings caused premature death in the zinc and copper deficiency treatments but no marked or characteristic symptoms except apparent phosphorus deficiency, could be discerned. Yields were reduced in all treatments by removal of the cotyledons, especially with copper deficiency. The growth of pea plants was depressed more by lack of zinc than of copper, but the latter deficiency resulted in complete prevention of seed formation (Plate II*a*). Relative effects on growth are shown by the yield data in Table I.

Copper deficiency.

Dwarf bean. Leaflets were dull faded green and became pale along major veins ; irregular necrotic areas appeared close to veins in central basal region of leaflets (Plate II*b*). Irregular marginal limpness and scorching (often on one side of the leaflet) was followed by withering and defoliation.

Runner bean. Symptoms appeared relatively suddenly towards the end of the experiment. Leaves were small, dull olive or yellow-green. Older leaves developed irregular orange-yellow intervenal blotching later wilting and withering. The shoot became chlorotic followed by die-back, and flowering was largely suppressed.

Broad bean. Habit erect and spindly. Leaflets were narrow, pointed and curled up at the tip. Wilting and withering of leaf tips followed. Red-brown necrotic and zoned areas developed and were apparently free from pathogens.

Pea. Growth was weaker and foliage paler than controls. Lower leaf scorch followed. Pods appeared normal and enlarged to normal shape but were entirely devoid of seed.

Alsike clover. Foliage was pale green ; leaflets were very small and juvenile in habit. Marginal or intervenal chlorosis was followed by wilting and finally withering ; flowering was suppressed.

Red clover. Foliage was pale, leaflets were very small and often withered after curling tip, wilting and scorching at margins and wilting in petioles ; flowering was suppressed.

Lucerne. Leaflets were very small ; older leaves were dark green, occasionally with marginal or intervenal orange blotches. Shoots wilted, young leaves remained folded, wilted and withered followed by death of the growing point and die-back, with development of basal axillary shoots ; flowering was suppressed.

Zinc deficiency.

Dwarf bean. Intervenal fading was followed by yellowing, defoliation of older leaves. Pods were shrivelled and not filled ; seeds failed to grow, or degenerated.

Runner bean. Foliage was dull olive green and leaflets small. Few pods formed, most shrivelled, seeds often degenerated and lacked cotyledons.

Broad bean. Foliage was pale and defoliated prematurely, especially when cotyedons were initially excised.

Pea. Foliage was pale dull green, older leaves withered.

Alsike clover. Foliage was pale, yellow green, orange or bronze tinted ; leaflet margins were wavy, with occasional crinkling of intervenal areas ; flowering was suppressed.

a. Effect of copper deficiency on pea.
Pods (left) externally normal and full size, but seed abortive.

b. Copper deficiency in dwarf bean ; basal necrotic spotting adjacent to major veins.

c. Zinc deficiency in red clover ; leaflets small, faded or chlorotic and tinted orange, later shrivelled ; margins often wavy and folded up at top.

d. Effects of different water supplies on copper deficiency in red clover.
Left to right : Control, metal stills, demineralised rain water, once glass distilled, twice glass distilled water.

PLATE II

Red clover. Foliage was pale green, fading around margins, later purpling, green along mid-rib and major veins in centre of leaflets ; margins were wavy and folded up or scorched. Fine dark brown necrotic spotting appeared in veins near margins, and as irregular necrotic intervenal areas ; flowering was suppressed (Plate II*c*).

Lucerne. Habit was erect and spindly ; leaflets were small, dull faded grey-green, or dark green with dark brown necrotic spots ; leaflets later showed orange tint in centres ; flowering was almost suppressed.

Sand and Water Test.

The differences due to sand treatment or water supplies were judged visually and by yields shown in Table I. There was no appreciable or consistent visual response due to sand treatment and on the whole a 2-day period appeared as satisfactory as a 3-day period, for copper or zinc deficiencies. Yield data showed a slight advantage with the 3-day treatment for copper deficiency in tomato but the reverse for clover. Water supplies on the other hand were clearly of critical importance. In the copper deficiency series, water from the metal stills (i) prevented acute deficiency from occurring. Tomato showed purple and yellow-purple tinting around and within the margins of older dark green leaves. Growth was not acutely depressed and the characteristic upcurling of the lamina recorded by Hewitt and Jones (1951), and with the other water supplies was absent. The effect of the metal distilled water was similar with red clover (Plate II*d*). Twice glass distilled water produced results in both crops slightly more acute than the once glass distilled water when judged by effects on yield. Demineralised rain water was almost comparable to once glass distilled water in the general severity of copper deficiency, and was consistently satisfactory over a period of over four months.

The overall effectiveness of technique for zinc deficiency appeared somewhat less efficient than for copper as found previously by Hewitt and Jones (1951). The effects of water supplies were, however, still apparent and similar to those noted for copper deficiencies, except that twice glass distilled water was not superior and was possibly inferior to once distilled water ; there was little difference between glass distilled and demineralised water supplies. Water from the tinned copper stills prevented the appearance of visual symptoms. There was some inconsistency in the behaviour of the replicates representing 2- or 3-day sand treatments in the zinc deficiency series. There was no obvious explanation except possibly contamination from condensation or paint falling on to the cultures.

Conclusions and Summary.

(1) Large seeded legumes (beans and pea) are less susceptible to copper deficiency than small seeded ones. Removal of cotyledons of dwarf bean or broad bean appreciably accentuated the effects.

(2) Dwarf bean was the most susceptible of the large seeded types and lucerne was the most susceptible of the small seeded types to omission of copper. Relative effects in the absence of zinc were less pronounced, and effect of seed size was appreciably outweighed by other factors.

(3) Duration of sand treatment did not consistently affect the incidence of either deficiency but water supplies were critical. Once or twice glass distilled water and demineralised rain water were practically similar. Water from tinned copper stills was markedly inferior and not satisfactory.

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INVESTIGATIONS ON POSSIBLE MICRONUTRIENT ELEMENTS FOR HIGHER PLANTS. I. EXPERIMENTS WITH COBALT, NICKEL AND GALLIUM IN SAND CULTURE.

By E. J. HEWITT and E. W. BOLLE-JONES.

The occurrence of cobalt in vitamin B_{12}, and its apparent requirement by species of *Euglena* and *Chlamydomonas* (8) and the reports that gallium may be an essential micronutrient for *A. niger* and *Lemna* (11), (12), indicated the need for further study of other possible micronutrient elements for higher plants. Although later work with gallium (9) (13) had negative results it was included with cobalt, and nickel, tested on grounds of its resemblance to cobalt, for the first trial. Both cobalt and nickel had previously been shown to be highly toxic to higher plants (1).

Experimental.

Methods.

Sand culture techniques fully described elsewhere (2) (5) (6) were used. Sand was treated with acid for four days and a detergent was included but preliminary sifting through metal sieves was omitted. Pyrex glass containers were rigorously cleaned in turn by a detergent in hot acid and alkaline solutions, concentrated acid and steaming. Water was first distilled from metal stills with silver condensers and then re-distilled twice from pyrex glass. M or 2M solutions were prepared from the A.R. grade re-crystallised nutrient reagents. These were adjusted to pH 2·8 and first extracted exhaustively with oxine and chloroform to remove gallium. 500 p.p.m. lead and copper were added, and precipitated by hydrogen sulphide, as tests had indicated substantial removal of cobalt (and to a lesser extent of nickel) by these means. After filtration on a sinter 56 mg. of purified ferric iron and an equivalent of re-crystallised oxine were added. The pH was raised to 6·5 by glass distilled ammonia solution and the precipitate filtered the following day. This step was based on an earlier procedure (4) for the removal of several micronutrients including gallium and also molybdenum. The alternative use of aluminium or of mixed organic reagents was not considered advantageous (10).

The use of an alkaline-earth phosphate precipitation was omitted although it was realised later that the method is particularly suited to the removal of aluminium, cobalt and nickel; these are in fact more efficiently removed than copper and zinc (7). Spectrographic tests also indicated that gallium was partly removed by an alkaline calcium phosphate precipitation (9).

The method used to purify ferric chloride and prepare ferric citrate resembled that already described (3). It was based on the use of oxine followed by 6N HCl and ether to remove gallium and molybdenum from a ferrous iron solution followed by extraction with 6N HCl and ether, after oxidation with hydrogen peroxide, to separate ferric iron from cobalt, nickel, manganese, copper, zinc and other metals.

Other micronutrient salts, except A.R. ammonium molybdate and "Specpure" gallium nitrate were re-crystallised twice with the addition of alcohol. The respective test elements were supplied at 0·005 p.p.m. together with the usual nutrients and 15 m.eq. L. nitrate nitrogen (2) (5) (6).

The crops grown were tomato, lettuce and sugar beet, chosen for the following reasons: Tomato is widely grown and its behaviour well known. It has a small seed and produces rapid and extensive vegetative growth. Lettuce has a small seed and matures rapidly. Sugar beet is a crop of unusual interest in relation to heavy metal toxicities (1) and the possible relation of cobalt to iron metabolism suggested its inclusion. Seeds were sown on 7th June, 1951, and germinated in about 10–12 days. Lettuce germinated badly and was re-sown without further difficulty. Plants were thinned at intervals to 1 per pot, or 2 per pot for sugar beet.

Results.

Lettuce. Apart from a uniformly distributed tendency to tip-burn, lettuce did not show any visual effects or treatment differences. Yields are recorded in Table I.

Sugar beet. All plants showed a faint mottling of middle leaves suggestive of mild iron deficiency. These effects persisted in middle and older leaves to varying degrees during the experiment and were most marked in the omitting nickel treatments. "Complete nutrient" treatment plants were slightly less vigorous than the others where one of the metals had been omitted. Yields are recorded in Table I.

Tomato. Plants made vigorous growth and during the earlier part of the experiment presented an exceptionally uniform appearance. They were in fact among the most vigorous and generally healthy-looking plants grown in sand cultures at Long Ashton during the last nine years. From the beginning of August, however, plants in the "Complete nutrient" treatment fell behind the others, at first almost imperceptibly and then quite obviously in general vigour and size of leaves, and in final yields as shown in Table I.

Toward the end of August plants in all treatments developed unfamiliar symptoms. Oldest leaves first showed a faint dull yellow, orange or purple tinted intervenal blotching suggestive of slight magnesium deficiency. This did not progress but old leaves (and progressively younger ones) curled upward along margins which became dark brown or almost black and necrotic especially at the extremities of segments. These symptoms were unlike any associated with known deficiencies but were similar in some respects to boron toxicity.

TABLE I.

MEAN YIELDS (G. DRY WT. PER PLANT) OF CROPS GROWN TO TEST EFFECTS OF COBALT, NICKEL AND GALLIUM IN SAND CULTURE

Crop	Harvest	Treatment	Shoot	Root	Fruit	Total
Tomato ..	Nov. 1st	"Complete"	71·95	22·75	88·3	183·0
		Omitting Ga	116·7	21·0	108·2	245·9
		,, Co	125·6	25·5	136·4	287·5
		,, Ni	120·6	31·2	132·8	284·6
Lettuce ..	Aug. 7th	"Complete"	8·40	0·78	—	9·18
		Omitting Ga	7·35	0·65	—	8·00
		,, Co	7·71	0·76	—	8·47
		,, Ni	7·05	0·43	—	7·48
	Aug. 21st	"Complete"	18·82	4·00	—	22·82
		Omitting Ga	20·06	4·58	—	24·64
		,, Co	16·39	3·70	—	20·09
		,, Ni	16·17	2·58	—	18·75
Sugar Beet ..	Aug. 7th	"Complete"	8·40	7·75	—	16·15
		Omitting Ga	12·20	5·45	—	17·65
		,, Co	15·63	7·92	—	23·55
		,, Ni	7·05	4·27	—	11·32
	Aug. 21st	"Complete"	27·50	21·5	—	49·00
		Omitting Ga	30·23	35·0	—	65·23
		,, Co	16·39	37·0	—	53·39
		,, Ni	27·87	26·0	—	53·87

From 7th September another symptom was observed. This affected mainly mid-stem leaves. It comprised at first a dull yellow-green paling of the upper surfaces of the basal-central region of leaflets, and was later tinted faintly brown. Leaf tips or areas adjacent to the midrib and major veins were occasionally involved and the under leaf surfaces were perceptibly paler. These effects were followed by marked brown necrosis in veins, along the midrib and lateral petioles, or in areas immediately adjacent to the midrib,

and visible on the under surfaces. Leaves curled down and leaflets curled backwards under the plane of the leaf.

These symptoms were detected in plants of all treatments but they were outstandingly more pronounced in plants of the "complete nutrient" treatment. The effects in the mid-stem regions were apparently free from complication by any pathogens and were again unlike anything previously associated with a mineral deficiency except for a certain resemblance to zinc deficiency recorded in 1950 (6) and confirmed this year. The latter, however, were not shown even when slight zinc contamination in the water maintained the supply above the threshold. Growth was greatly in excess of that at which visual symptoms of zinc deficiency appear.

Moreover the micronutrient supplements (4 in all) were all provided with zinc and were used without change during the whole course of the experiment. Milder symptoms of leaf marginal curl and necrosis of old leaves also occurred in other tomato plants given an apparently complete nutrient, less rigorously purified sand and supplied with demineralised water, but the mid-stem leaf effects here were not seen.

Further discussion of these results would be premature at this stage except to point out certain facts.

First ; the symptoms in tomato although unusual were not entirely distinct from effects known to be due to a mineral excess, and a mineral deficiency.

Second ; the symptoms were not restricted to a known omission of the three elements –cobalt, nickel or gallium—but were apparently more marked when all three were known to be present.

Third ; there was no evidence that these elements were essential, but if so then the thresholds of deficiency occur probably at very low concentrations.

Experience with copper and zinc deficiencies, however, has shown that consistent elimination of amounts below 0·001 p.p.m. may be difficult compared with the removal of molybdenum to 0·00005 p.p.m. and in fact often one-tenth of this. Thus there is at present no guide as to what lower limits of concentration might have been attained until analytical data on dried foliage and culture materials are available.

The possibility that some other previously unrecognised micronutrient element might have been deficient in tomato cannot be ruled out at present. Tomato seed was saved with the intention of re-sowing in 1952 to test this possibility and to repeat the work recorded here.

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THE EFFECTS OF INTER-RELATIONSHIPS BETWEEN MOLYBDENUM AND NITROGEN SUPPLY ON THE FREE AMINO-ACID STATUS OF CAULIFLOWER PLANTS GROWN IN SAND CULTURE.

By S. C. AGARWALA and A. H. WILLIAMS.

Hewitt, Jones and Williams (1), using a paper chromatographic technique applied to extracts of cauliflower leaves, showed that the content of free amino acids was reduced at low levels of molybdenum when nitrate was used as a source of nitrogen. Later work (2) showed that when ammonium nitrogen was given, cauliflower and tomato leaves contained abundant amino acids whether molybdenum was given or excluded from the nutrient solution.

Cauliflower (var. Majestic) was grown in sand culture at deficiency levels of Mo of 0·00001 and 0·00005 p.p.m., a normal level of 0·5 p.p.m. and an excess level of 50 p.p.m., with nitrogen at 10 m.M./L as follows : (i) nitrate without calcium carbonate ; (ii) nitrate with calcium carbonate ; (iii) nitrate with citric acid ; (iv) ammonium sulphate with calcium carbonate ; (v) ammonium citrate ; (vi) ammonium nitrate ; (vii) urea ; and (viii) nitrite. Six gm. leaf material was collected from the two blocks and extracted in 70% alcohol, concentrated in vacuo and examined by paper partition chromatography using phenol/water, or Butanol/acetic acid/water as solvents. The relative concentrations of amino acids were assessed visually on area and intensity of the spots produced after reaction with ninhydrin. The extracts were tested at different concentrations and assessed independently. The concentrations were compared in three ways :—

(a) With respect to effect of molybdenum status on individual amino acids for each source of nitrogen in turn (Table I).

(b) With respect to different nitrogen sources at each level of molybdenum.

(c) With respect to the relative concentration of the various amino acids for each nitrogen source and level of molybdenum (Table II).

TABLE I. The effect of molybdenum status on the concentration of amino acids.

Nitrogen at 10 m.M/L as :—	Nitrate				Nitrate + $CaCO_3$				Nitrate + Citric acid				Ammonium Sulphate + $CaCO_3$				Ammonium Citrate				Ammonium Nitrate				Urea				Nitrite			
Molybdenum p.p.m.	0·00001	0·00005	0·5	50·0	0·00001	0·00005	0·5	50·0	0·00001	0·00005	0·5	50·0	0·00001	0·00005	0·5	50·0	0·00001	0·00005	0·5	50·0	0·00001	0·00005	0·5	50·0	0·00001	0·00005	0·5	50·0	0·00001	0·00005	0·5	50·0
(A) Aspartic	t	t	M	M	t		M	M	S	W	M	M	t	W	M	M	t	t	W	t	t	t	M	M	t	t	M		t		S	M
(B) Glutamic	W	WM	MS	MS	W		MS	MS	S	S	M	M	W	W	M	M	W	t	M	M	W	W	M	M	W	W	M		W		MS	MS
(C) Serine	t	M	W	W	t		t	S	M	M	W	M	W	M	W	W	S	S	M	M	M	W	M	MS	S	W	M		t		MS	M
(D) Asparagine	t	M	W	W	t		t	W	M	M	W	W	W	M	W	W	W	W	W	M	t	t	M	S	S	W	M		t		W	M
(E) Threonine	t	M	W	W	t		t	W	M	M	W	W	W	M	W	W	W	W	M	M	t	t	M	M	S	W	M		t		M	M
(F) Glutamine	W	M	S	S	M		MS	S	S	M	M	M	W	M	MS	MS	W	W	S	MS	WM	WM	S	S	S	S	S		M		S	S
(G) Unknown	W	M	W	W	t		W	S	M	t	t	W	W	W	M	M	W	W	M	M	W	t	W	M	S	WM	W		M		W	M
(H) Arginine	t	M	t	t	WM		t	W	MS	t	t	W	MS	M	W	W	S	W	W	W	S	WM	M	M	VS	S	MS		t		MS	M

Samples spoiled

All plants killed

Samples spoiled

t = trace ; W = Weak ; WM = Weak Medium ; M = Medium ; MS = Medium Strong ; S = Strong ; VS = Very Strong→Arbitrary terms to indicate the differences between different nitrogen sources or between different amino acids.

TABLE II. The concentration of amino acids relative to each other with each treatment.

Nitrogen at 10 m.M/L as :—		Nitrate				Nitrate + $CaCO_3$				Nitrate + Citric acid				Ammonium Sulphate + $CaCO_3$				Ammonium Citrate				Ammonium Nitrate				Urea				Nitrite			
Molybdenum p.p.m.		0·00001	0·00005	0·5	50·0	0·00001	0·00005	0·5	50·0	0·00001	0·00005	0·5	50·0	0·00001	0·00005	0·5	50·0	0·00001	0·00005	0·5	50·0	0·00001	0·00005	0·5	50·0	0·00001	0·00005	0·5	50·0	0·00001	0·00005	0·5	50·0
Highest	I	F	F	F	F	F		F	F	F	F	F	F	F,H	F	F	F	H	H	F	F	H	F	F	F	H	H	F		F		F	F
	II	B	H	B	B	B		B	B	B	B	B	B	C	H	H	H	F	F	H	H	F	C	H	C	F	F	H		B		B	B
	III	EH	B	C	C	H		A,C, D,E, G	E,H	H	E	E	E	B	C	B	B	C	C	C	B,C	C	B	C	H	C	C	C		A,C, D,E, G,H		C	C
decreasing order	IV	C	C	E	E	A,C, D,E, G		H	C	E	C	C	C	E	B	C	C	B	E	B	E	B	A,D, E,G, H	B	E	E	B	B		E		E	E
	V	D	E	D	A				D	C	D	D	D	D	E	E	E	E	D	E	D	E		E	B	D	E	E		H		H	H
	VI	A	D	H	D				A	A,D	A	A	A	A	D	D	D	D	B	D	A	G		D	D	A,B	D	D		D		D	D
	VII	G	A	A	G				G	G	H	H	H	G	A	G	G	G	G	G	G	D		A	A	G	G	G		A		A	A
Lowest	VIII		G	G	H						G	G	G		G	A	A	A	A	A	A	A		G	G		A	A		G		G	G

Samples spoiled

All plants killed

Samples spoiled

The last assessment, in addition to being subjective like the rest, was also based on the assumption that equal concentration of α-amino groups produced equal areas and intensities of colour on reaction with ninhydrin irrespective of the amino acid concerned. In this respect, therefore, only major differences in intensity can be admitted and this reservation has been adopted here.

Results.

The effects of molybdenum status with different nitrogen sources on growth, ascorbic acid and development of " Whiptail " have been reported elsewhere (3). This is mentioned here, however, as the results obtained gave a clear indication that molybdenum was required with all nitrogen sources though least with nitrite.

The following amino acids and amides were found : (A) aspartic acid, (B) glutamic acid, (C) serine, (D) asparagine, (E) threonine, (F) glutamine, (G) unknown, and (H) arginine. Irrespective of the molybdenum level and nitrogen source, the two amides glutamine and asparagine were found in higher concentrations than their respective amino acids. The concentrations of glutamine and glutamic acid were always higher than those of asparagine and aspartic acid respectively.

At the normal molybdenum level with all nitrogen sources glutamine was the principal amino acid but nitrogen source determined which amino acid occupied the second place. Thus, the second place was taken by glutamic acid with nitrate and nitrite types, while arginine occupied the second place with ammonium and urea compounds. At the deficiency levels of Mo as compared with the normal, the concentration of glutamic acid decreased with all nitrogen treatments except with nitrate plus citric acid. Considerable accumulation of arginine took place with urea at 0·00001 and 0·00005 p.p.m. Mo and with ammonium nitrate and ammonium citrate at 0·00001 p.p.m. Mo, with the result that arginine became the principal amino acid in these treatments. The effect was specially pronounced with urea. With ammonium sulphate plus calcium carbonate also, marked accumulation of arginine took place at the deficiency levels of Mo but arginine became the principal compound only at 0·00001 p.p.m. Some accumulation of arginine was also found at 0·00005 p.p.m. Mo with nitrate alone and at 0·00001 p.p.m. Mo with nitrate plus calcium carbonate.

Supply of citric acid with nitrate increased the concentration of amino acids at the deficiency level of Mo but not at the normal level of Mo, with the result that the concentration of amino acids at the deficiency levels of Mo became higher than at normal level (with the possible exception of aspartic).

Interaction of nitrogen source with excess molybdenum did not show any marked changes in the amino acid concentrations.

Discussion.

The results presented here are at variance with the earlier observations (2) in that at the deficiency level of Mo as compared with normal molybdenum level with ammonium nitrogen source glutamic acid was clearly depressed and arginine accumulated whereas previously no consistent or marked differential concentration effects were observed.

From the results presented here it is evident that depression of glutamic acid at the deficiency level of Mo was independent of nitrogen source. Supply of citric acid with nitrate reversed this effect. This and the fact that at normal molybdenum level the supply of citric acid with nitrate, as compared with nitrate only, did not increase the amino acid concentration would suggest that in plants grown with nitrate but without adequate molybdenum, lack of carboxylic acids was a greater limiting factor in the formation of amino acids, than the supply of assimilable nitrogen.

The increase of amino acid concentration at the deficiency level of molybdenum with nitrate plus citric acid did not alleviate the visual symptoms of molybdenum deficiency, in fact, the Whiptail effect was accentuated in this treatment. Citrate ion in ammonium citrate did not produce the same reaction as citric acid with nitrate in changing the amino acid concentration at the deficiency level of Mo but " Whiptail " still occurred. On the other hand the deficiency symptoms were least marked with nitrite (3), but the amino acid status was still markedly affected.

At the normal level of molybdenum, ammonium and urea, as contrasted with nitrate, produced an accumulation of arginine. This tendency became pronounced with the deficiency of molybdenum. With urea, accumulation of arginine was particularly great. Formation of urea from arginine is a normal event in many animals (4). The reverse process, i.e. formation of arginine from urea has not so far been reported for any organism. In view of the fact that ornithine was not detected in cauliflower, this possibility seems remote but cannot be entirely ignored.

The different amino acid patterns found with different nitrogen sources support the earlier conclusion (3) that ammonium and urea nitrogen supplied in sand culture entered cauliflower without appreciable nitrification. Steinberg (5, 6) has shown that " frenching " in tobacco could be produced by supplying isoleucine to culture medium and has suggested that the visual symptoms characteristic of individual mineral deficiencies may be due to an accumulation of a particular amino acid. The symptoms characteristic of molybdenum deficiency cannot be easily explained on that basis. It would, however, be of interest to determine whether the visual injury effects noted with ammonium and urea–N are due to the accumulation of arginine, and whether the extra accumulation of this acid at the deficiency levels of Mo is responsible for leaf malformation.

Summary.

Depression of glutamic acid at the deficiency level of molybdenum was found to be independent of nitrogen source. Supply of citric acid with nitrate reversed this effect. Arginine became an important amino compound with ammonium and urea–N supply and this effect was pronounced at the deficiency level of molybdenum with these N–treatments where arginine generally became the principal amino compound.

Acknowledgment.

We wish to thank Dr. E. J. Hewitt for his criticism and helpful suggestions.

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THE EFFECT OF MOLYBDENUM AND NITRATE STATUS ON THE CARBOHYDRATE AND NITROGEN METABOLISM OF CAULIFLOWER PLANTS IN SAND CULTURE.

By S. C. AGARWALA.

In a previous paper (1) the effect of molybdenum status and nitrate supply on growth and ascorbic acid concentration has been described. This paper summarises the effect of molybdenum status and nitrate supply on chlorophyll and some nitrogen and carbohydrate fractions.

Methods.

Material for analysis was obtained from plants grown at eight levels of molybdenum ranging from 0·000005 to 19·2 p.p.m. and at two levels of nitrate nitrogen, 6 and 24 mg.eq. l. The culture technique was described previously (1).

Molybdenum. The method of Piper and Beckwith (2) was used.

Chlorophyll. The method of Comar *et al* (3) as modified at Long Ashton for small samples (4, 5) was used.

Carbohydrates. No starch was detected by iodine even when examined microscopically.

Sugars were determined on 80% alcoholic extracts. Chromatographic examination made on de-ionised alcoholic extracts showed the presence of sugars other than glucose, fructose and sucrose. In order to characterise the unknown sugars separations were made on a column of powdered cellulose at 65° C. using n-butanol, half saturated with water at room temperature, as the eluting agent according to the method of Counsell *et al* (6). The sugar with the lowest R.F. value did not come out even after 10 days continuous run and had to be washed out of the cellulose column with water.

Glucose and fructose were determined quantitatively on one set after separation on a paper chromatogram and extraction of sugars in a micro Soxhlet's apparatus, by the method of Nelson (7).

Total reducing sugars and oligosaccharides easily hydrolysed with dilute acid were determined by the method of Nelson (7).

Nitrogen fractions. Nitrite and hydroxylamine were determined on Morgan's extract using Greiss-Illoway reagent.

Ammonia was also determined on Morgan's extract by the method of Wolf (8) or by the method of Conway (9) as modified by Hunter (10).

Nitrate was determined both on Morgan's extract of fresh material or on oven dried material by the method of Richards and Templeman (11). Only the results on dried material are presented here.

Total nitrogen was determined by a semi-micro Kjeldahl method (12). Salicylic acid was used to include the nitrogen fraction in the estimation (13). Use of the iron reduction method (14) for the purpose was found to give low values.

Results.

Molybdenum status.

Irrespective of molybdenum and nitrate supply the concentration of molybdenum was found to be greatest in leaves followed in decreasing order in roots, stems and curds. The concentration in roots was in many instances nearly the same as in leaves. The concentration of molybdenum in leaves during August and September did not increase with an increase in the molybdenum supply from 0·000005 to 0·0005 p.p.m. During December there was a slight increase at 0·0005 p.p.m. Increase of molybdenum supply beyond 0·0005 p.p.m. resulted in increased molybdenum concentration in plants, but the increase was not proportional to molybdenum supply.

With a molybdenum supply of 0·48 p.p.m. and above the concentration in plants seemed to depend on nitrate supply, accumulation being greater with 24 mg.eq./l. nitrate supply than with 6 mg.eq./l.

It was previously reported that plants grown with excess molybdenum showed " blue patches " in leaves and according to Warington (15) the blue colour is due to an anthocyanin-molybdenum complex. The molybdenum content of " blue areas " and normal areas of the same leaf did not show any consistent difference. The concentration of molybdenum in both the blue and normal areas was excessive and attained a level of 7,000–8,000 p.p.m., i.e. 0·7 to 0·8 per cent. dry matter.

Chlorophyll.

The concentration of chlorophyll in mature leaves supplied with 0·000005 and 0·00005 p.p.m. molybdenum was less than in plants supplied with 0·0005 to 19·2 p.p.m. In young leaves, however, the molybdenum effect was negligible.

The concentration of chlorophyll in leaves of plants supplied with high nitrate was higher than in those supplied with low nitrate except in a few cases at the lowest molybdenum level and during July when no marked or consistent differences were found in plants supplied with different levels of nitrate nitrogen.

The chlorophyll concentration in leaves of plants deficient in molybdenum decreased with maturity whereas in plants with normal or excess molybdenum supply chlorophyll increased.

Carbohydrates.

No starch was detected in cauliflower. Some of the data obtained are given in Table III or total and reducing sugars in leaves and stems. Stems contained more of both total and reducing sugars than leaves. Glucose and fructose comprised the bulk of sugars and were present in almost 1:1 ratio, but fructose was slightly in excess.

TABLE I.

Molybdenum Present (as p.p.m. in Dry Material) in Cauliflower Plants Grown at Different Molybdenum and Nitrogen Levels.

Mo supplied	NO_3 m.eq./L. supplied	August	September	December—January			
		Leaves	Leaves	Leaves	Stems	Curds	Roots
0·000005	6	0·08	0·07	0·11	0·04	No Curd	0·09
0·000005	24	0·06	0·05	0·15	0·05	„ „	0·06
0·00005	6	0·08	0·04	0·24	0·05	„ „	0·12
0·00005	24	0·11	0·06	0·09	0·06	—	0·11
0·0005	6	0·11	0·10	0·53	0·21	0·62	0·42
0·0005	24	0·13	0·06	0·50	0·12	0·45	1·87
0·005	6	1·12	0·48	1·35	0·18	0·31	0·68
0·005	24	1·39	0·20	1·54	0·19	0·29	0·68
0·05	6	5·0	1·47	1·46	0·47	0·91	0·96
0·05	24	4·82	1·92	5·12	1·25	0·79	3·3
0·48	6	19·36	12·07	19·2	1·60	3·51	5·84
0·48	24	57·20	29·89	42·5	7·30	7·58	129·0
4·8	6	341·5	50·0	197·2	23·49	26·36	347·8
4·8	24	572·0	429·8	554·3	75·73	41·67	308·8
19·2	6	1,458·0	1,915·0	683·5	197·30	54·30	697·2
19·2	24	2,391·0	3,924·0	2,357·5	496·75	222·05	1,232·0

Leaves showing blue patches (molybdenum-anthocyanin complex).
Mo supplied 19–78 p.p.m.

Normal Areas.	Blue Areas.
4562	4697
3440	3853
2852	1518
7455	2530
5076	8085
Mean 4846	4146

In addition to the sugars mentioned above and sucrose, galactose, probably ribose, another pentose with a lower R.F. value than ribose and three oligosaccharides were present. These three had the following respective characteristics : (i) gave glucose, galactose and fructose on acid hydrolysis ; it was non-reducing and was not hydrolysed by invertase and therefore raffinose was excluded ; (ii) gave glucose and galactose and was a reducing disaccharide ; (iii) gave glucose and galactose and was non-reducing.

Final characterisation is in progress. Chromatographic studies indicated that all the sugars were decreased with lack of molybdenum.

TABLE II.

Chlorophyll mg./100 gm. Fresh Weight, in Cauliflower Plants Grown at Different Molybdenum and Nitrogen Levels.

Mo supply p.p.m.	Nitrate supply	July	August	September	September	December
		Middle Leaves	Middle Leaves	Middle Leaves	Young Leaves	Middle Leaves
0·000005	6	109·5	70·0	51·0	115·0	50·3
0·000005	24	76·5	41·0	39·0	124·5	69·0
0·00005	6	110·5	89·5	59·5	124·5	85·0
0·00005	24	84·0	86·5	92·3	184·5	151·0
0·0005	6	138·0	147·5	139·5	137·5	112·0
0·0005	24	142·5	168·0	200·0	170·5	151·5
0·005	6	150·5	109·5	136·0	—	124·5
0·005	24	141·0	151·5	178·5	—	130·5
0·05	6	—	138·0	167·0	—	122·0
0·05	24	144·0	153·5	203·0	—	165·0
0·48	6	132·0	130·5	153·5	115·0	135·5
0·48	24	144·5	138·5	211·5	133·0	197·0
4·8	6	128·5	155·0	142·0	—	178·0
4·8	24	127·0	153·5	216·5	—	182·0
19·2	6	128·0	135·0	124·0	—	151·0
19·2	24	109·0	125·0	144·5	—	200·0

Table III shows not only the overall effect of molybdenum status on sugars but reveals also that in leaves there were reversal effects from 0·000005 to 0·0005 p.p.m Mo at high nitrate level and from 0·000005 to 0·00005 p.p.m. at low nitrate evel.

Nitrogen fractions.

Nitrite and hydroxylamine were just detectable in some samples. The presence of nitrite and hydroxyl-amine was not found to bear any relation to molybdenum status. The maximum level found did not exceed 0·1 p.p.m.

Ammonia. Low concentrations of ammonia, ranging from 25 to 75 p.p.m., were found in leaves of plants grown at different levels of molybdenum and nitrate. The concentrations of ammonia was not related to molybdenum status.

Total nitrogen. With a few exceptions the concentration of total nitrogen in plants with high nitrate supply was more than in plants with low nitrate supply and molybdenum status did not show much effect on the concentration of total nitrogen whereas the relative concentration of nitrate and organic nitrogen fractions varied greatly with molybdenum supply as shown below.

TABLE III.

Effect of Molybdenum Status on Sugar Content in Cauliflowers Grown at Different Nitrogen Levels (Values as Per Cent. of Fresh Weight). R.S. = Reducing Sugars; T.S. = Total Sugars; G.F. = Glucose/Fructose ratio.

Mo level p.p.m.	Nitrogen level m.eq./L.	Leaves					Stems	
		September		December			December	
		R.S.	T.S.	R.S.	T.S.	G.F.	R.S.	T.S.
0·000005	6	0·54	0·54	0·43	0·45	1/1·08	0·95	1·05
	24	0·29	0·30	0·28	0·30	1/1·10	0·65	0·70
0·00005	6	0·30	0·30	0·27	0·29	1/1·02	1·04	1·04
	24	0·66	0·69	0·20	0·26	1/0·98	0·78	0·80
0·0005	6	0·81	0·84	0·725	0·75	1/1·12	1·14	1·18
	24	0·84	0·88	0·175	0·20	1/1·3	1·175	1·26
0·005	6	0·78	0·92	0·785	0·82	1/1·10	1·55	1·60
	24	0·96	0·96	0·825	0·84	1/1·20	1·50	1·80
0·05	6	0·805	0·82	1·02	1·30	1/0·98	1·52	1·56
	24	0·915	0·96	1·25	1·32	1/0·94	0·925	0·98
0·5	6	0·85	0·86	0·80	0·86	1/1·30	1·555	1·59
	24	0·88	0·90	0·50	0·65	1/1·20	1·35	1·41
4·8	6	0·87	0·91	0·825	1·10	1/1·40	1·30	1·30
	24	0·96	0·96	0·42	0·50	1/0·96	1·70	1·70
19·2	6	0·975	0·99	0·775	0·81	1/1·40	1·85	1·88
	24	missing		0·95	1·20	1/0·95	missing	

Nitrate. Accumulation of nitrate varied in different parts of the plant and depended on both molybdenum and nitrate supply.

For any molybdenum level, leaves showed the highest nitrate accumulation followed in descending order by stems, roots and curds. Deficiency of molybdenum caused accumulation of nitrate in all parts except curds. The effect was most pronounced in leaves and only slight in roots. A maximum of 17·7% nitrate, i.e. 3·86% nitrate nitrogen was found at 0·000005 p.p.m. molybdenum with high nitrate supply during December. In leaves and roots nitrate accumulation fell sharply to 0·0005 p.p.m. molybdenum, beyond which the effect was not consistent. In some samples there seemed to be an appreciable response even at the highest molybdenum level. With the high nitrate supply there was, during July and December in some samples, as much nitrate nitrogen at 4·8 and 19·2 p.p.m. molybdenum as with low nitrate supply and 0·000005 and 0·00005 p.p.m. molybdenum.

Organic nitrogen. Organic nitrogen was calculated as the difference between

TABLE IV.

Total Nitrogen (T.N.), Nitrate Nitrogen (N.N.) and Organic Nitrogen (O.N.) as Per Cent. Dry Matter in Cauliflower Plants Grown at Different Molybdenum and Nitrogen Levels.

Molybdenum supply p.p.m.	Nitrate supply mg.eq./L.	JULY			AUGUST			SEPTEMBER			DECEMBER											
		Leaves			Leaves			Leaves			Leaves			Stem			Curd			Roots		
		T.N.	N.N.	O.N.	T.N.	N.N.	O.N.	T.N.	N.N.	O.N.	T.N.	N.N.	O.N.	T.N.	N.N.	O.N.	T.N.	N.N.	O.N.	T.N.	N.N.	O.N.
0·000005	6	3·72	0·48	3·24	3·49	0·80	2·69	2·66	1·95	0·71	3·92	2·39	1·53	1·66	0·92	0·73				2·40	0·15	2·25
0·000005	24	6·18	3·45	2·73	5·34	1·60	3·74	4·01	2·10	1·91	5·90	3·86	2·04	2·37	0·95	1·45	No Curd			1·55	0·33	1·22
0·00005	6	3·20	0·11	3·09	4·69	0·80	3·89	2·88	1·60	1·28	3·96	2·08	1·88	2·33	0·90	1·43				2·22	0·22	2·00
0·00005	24	5·64	2·01	3·63	4·28	1·49	2·79	3·63	1·28	2·35	4·49	2·09	2·40	1·40	0·57	0·83	—	0·002	—	2·21	0·28	1·93
0·0005	6	4·73	0·17	4·56	3·55	0·05	3·50	2·53	0·24	2·39	2·77	0·24	2·53	2·45	0·17	2·28	5·45	0·002	5·45	1·21	0·01	1·20
0·0005	24	5·10	1·33	3·77	5·45	0·37	5·07	5·32	0·30	5·02	4·33	1·85	2·48	2·94	0·59	2·35	4·47	0·003	4·47	1·37	0·14	1·23
0·005	6	3·93	0·17	3·76	5·30	0·08	5·22	1·45	0·04	1·41	2·24	0·17	2·07	1·70	0·11	1·59	3·23	0·002	3·23	1·80	0·03	1·77
0·005	24	5·92	0·95	4·97	5·66	0·45	5·20	2·60	0·13	2·47	3·97	1·16	2·81	2·63	0·58	2·05	4·72	0·004	4·72	2·36	0·11	2·25
0·048	6	5·59	nil	5·59	5·91	0·08	5·83	2·37	0·04	2·33	3·25	0·14	3·11	0·96	0·26	0·70	5·38	0·005	5·37	1·47	0·03	1·44
0·048	24	5·79	0·71	5·08	5·49	0·24	5·24	2·59	0·32	2·38	4·01	1·17	2·84	2·06	0·36	1·70	3·82	0·008	3·81	1·70	0·06	1·64
0·48	6	4·93	0·04	4·89	4·32	0·07	4·25	1·93	nil	1·93	3·28	0·02	3·26	1·11	0·15	0·96	4·17	0·005	4·16	1·12	0·03	1·19
0·48	24	6·02	1·35	4·67	5·25	0·24	5·01	2·82	0·05	2·75	3·69	1·18	2·51	2·90	0·62	2·28	4·14	0·005	4·13	2·30	0·12	2·18
4·8	6	4·53	0·11	4·42	5·29	0·05	5·24	1·87	nil	1·87	2·80	0·19	2·61	1·85	0·29	1·56	4·06	0·005	4·05	1·38	0·10	1·28
4·8	24	5·24	0·68	4·56	5·44	0·27	5·17	3·31	0·12	3·19	3·91	1·51	2·40	2·49	0·55	2·94	3·34	0·003	3·34	2·26	0·14	2·12
19·2	6	4·00	0·11	3·89	5·04	0·09	4·95	2·24	0·04	2·20	2·41	0·26	2·15	0·95	0·15	0·80	5·33	0·013	5·32	1·41	0·04	1·37
19·2	24	5·18	0·73	4·45	5·62	0·17	5·45	2·88	0·20	2·68	3·23	0·74	2·49	1·80	0·58	1·22	4·60	0·003	4·60	1·80	0·16	1·64

total nitrogen and nitrate nitrogen. Inorganic nitrogen other than nitrate was found to be negligible and therefore no allowance has been made for it in the calculation of organic nitrogen.

Deficiency of molybdenum, i.e. levels of 0·000005 and 0·00005 p.p.m., lowered the concentration of organic nitrogen in leaves both with high and low nitrate supply. Molybdenum status did not show any consistent effect on the concentration of organic nitrogen in stems, curds and roots. In some instances the concentration of organic nitrogen in plants supplied with low nitrate was more than in plants supplied with high nitrate, although the latter gave higher yields.

Summary and Conclusions.

Plants grown with eight levels of molybdenum ranging from 0·000005 to 19·2 p.p.m. and with nitrate supply of 6 and 24 mg.eq. l. were analysed for molybdenum, chlorophyll, sugars, nitrite, hydroxylamine, ammonia, nitrate and organic nitrogen.

The following conclusions are drawn from the data :—

(1) Molybdenum accumulation was greatest in leaves and roots which are regarded as probable sites of nitrate reduction in plants.

(2) The concentration of molybdenum in plants supplied with 0·000005 to 0·0005 p.p.m. molybdenum showed little difference although yield progressively increased at levels from 0·000005 p.p.m. to 19·2.

(3) With a molybdenum supply of 0·48 p.p.m. or more, the high nitrate plants accumulated more molybdenum than the low nitrate plants.

(4) Leaves showing "blue patches," presumably an anthocyanin-molybdenum complex, showed great accumulation of molybdenum.

(5) Lack of molybdenum depressed the chlorophyll content of mature leaves but not of young leaves.

(6) The concentrations of various sugars found in cauliflower were depressed at the low levels of molybdenum and showed a minimum at 0·00005–0·0005 p.p.m. Mo, whereas chlorophyll content increased steadily from the lowest level of molybdenum—0·000005 p.p.m. Lack of chlorophyll alone, would not, therefore, seem to account for the effect on sugar content and the demand for these for nitrate assimilation might be partly responsible for the results obtained.

(7) Nitrate accumulated at the deficiency level of molybdenum and decreased rapidly to 0·0005 p.p.m. molybdenum beyond which no consistent effects were noted.

(8) Accumulation of nitrate did not seem to be responsible for the symptoms characteristic of molybdenum deficiency as, during July and December with high nitrate, there was as much residual nitrate at 4·8 and 19·2 p.p.m. Mo, as with low nitrate supply at 0·000005–0·00005 p.p.m. molybdenum, although there were no injurious effects in the former.

(9) The concentration of organic nitrogen in leaves decreased at the deficiency levels of molybdenum.

(10) The concentration of nitrite, hydroxylamine and ammonia did not bear any relation to molybdenum status.

(11) The results indicate that the role of molybdenum may concern processes more fundamental than the reduction of nitrate.

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THE MANGANESE STATUS OF CEREAL CROPS IN RELATION TO YIELD OF GRAIN AND STRAW. I. FIELD EXPERIMENT ON OATS. 1949 and 1950.

By D. J. D. NICHOLAS and D. J. FISHER.

The characteristic symptom of " grey speck " in oat is as well known as is the use of foliage sprays of manganese sulphate for its control (3, 4, 7, 17, 19, 20, 25, 26). Other corrective methods for Mn deficiency in crops include the addition of sulphur or thiosulphates (18) to Mn deficient soils, which may result in an increase in available Mn. A detailed study of the oxidation and reduction of Mn in soils, brought about by chemical and microbiological processes has been made by Lees and Quastel (10) and Mann and Quastel (12), Sherman *et al.* (21, 22) and Leeper (9).

Numerous workers have related symptoms of Mn deficiency in oat to the low Mn content of the plants (9, 13, 17, 18, 20) whereas others failed to find a consistent difference between normal and deficient plants (5, 11, 24). Also (1), Leeper (9), Wallace and Ogilvie (25), Gallagher and Walsh (3, 4), Barclay (2) and Quastel *et al.* (18) have reported better growth and increased yields of crops following manganese treatment.

Little information is available on the relation between Mn status of crops and final yields. Goodall (8) has shown that the Mn content of winter wheat can be used to forecast its response to foliage sprays of manganese sulphate. The Mn contents of wheat above which no further increase of grain can be expected, as a result of increasing the element, were determined as follows : during tillering 34 p.p.m. in dry matter of lower leaf blades and 36 p.p.m. in dry matter of stems prior to leaf emergence (8).

In this preliminary paper it is shown that foliage sprays of $MnSO_4$ at 0.1, 0·25 and 0·5% respectively were far more effective in correcting Mn deficiency in oat than placing S at 2, 4 and 8 cwt., per acre respectively with the seed. Placing $MnSO_4$ with the seed at 96 lb. per acre, although better than S alone in overcoming the deficiency in oat, was not as effective as foliage sprays of $MnSO_4$.

In addition an attempt has been made to correlate the Mn contents of the mid-stem leaves, determined at intervals during the season, with final yields. The relations between K, Ca, Mg, P and Mn have been studied statistically. It is shown that there is a significant relation between Mn content of the mid-stem leaves and final yields during early season but that later there is no correlation. The Mn status is more closely related to the visual symptoms at this stage of growth. Critical levels for Mn in mid-stem leaves, over and above which a response to further applications of Mn would not be expected, are suggested for the early stages of growth. The determination of critical levels is however complicated by the presence of residues of $MnSO_4$ on the leaves in the spray treatments, and the relative ineffectiveness of soil methods for correcting the deficiency.

Experimental.

The site chosen for the experiments was at Hanham in Bristol. The soil is a sandy black loam derived from Pennant Grit and has been subjected to intensive cultivation as a market garden for a number of years. The surface soil, which contains free calcium carbonate, has a pH of 7·1. The exchangeable, easily reducible and total Mn are 1·1, 31, and 341 p.p.m. respectively. Most vegetable and fruit crops showed severe symptoms of Mn deficiency at this centre (15).

Oat var. S 84 was grown as the test crop in 1949 and 1950. The seed was drilled at the rate of 4 bushels per acre.

Experimental Layout.

As this is a private garden, space did not allow for factorial experiments ; two Latin squares were therefore used.

Experiment 1 (1949). The following treatments were given : foliage sprays at 0, 0·1, 0·25 and 0·5% $MnSO_4$ respectively at the rate of 200 gall. per acre. These were applied five times during the season, on 19th and 29th April, 9th, 19th and 30th May.

Experiment 2 (1949). In an adjacent Latin square sulphur was placed with the seed at 0, 2, 4 and 8 cwt. per acre respectively.

Experiments 3 *and* 4 (1950). During 1950 placement experiments with the seed were carried out in two Latin squares. The treatments were nil ; $MnSO_4$ at 96 lb. per acre ; S at 4 cwt. per acre ; and $MnSO_4$ + S at the same rates as for the previous treatments.

Plant Sampling and Analysis.

Experiment 1. Mid-stem leaves were sampled from all plots five times during the season on the 9th, 19th, 30th May, 9th and 20th June.

Tissue tests for K, Mg, Ca, P, nitrate N, and Mn were made on the fresh mid-stem leaves and the mineral elements, other than nitrate N, were also

A

B.

A. *Experiment* 2 1949 (oat)
Sulphur placement treatments at 2, 4 and 8 cwt. per acre; severe Mn deficiency in all plots.

B. *Experiment* 1 1949 (oat)
Foreground—Foliage spray treatments of $MnSO_4$, growth vigorous; control plots, marked chlorosis (Mn deficiency).
Background—Sulphur placement treatments, severe Mn deficiency.

Fig. 1.

determined in the ash (13, 14, 15). The tetrabase method (14, 16) was used on acid digests of the plants to determine deficiency levels of Mn below 20 p.p.m. in dry matter. The periodate method (16) was used for higher values.

Results.

Visual Symptoms.

Experiments 1 *and* 2 (1949). Grey speck symptoms appeared in plants in the nil plots and those given S only, a month after seeding. Plants given foliage sprays were normal at this stage; no differences appeared following the various rates of Mn spray treatments. Two weeks later, the non-treated plants and those given S at the three rates showed marked chlorosis and withering of the older leaves as shown in Fig. 1 (Plate III). Symptoms of Mn deficiency appeared in the mid-stem leaves of the Mn spray treatments at the end of June, after spraying had been discontinued.

Experiments 3 *and* 4 (1950). Symptoms appeared 5 weeks after drilling the seed in the nil plots and in those given S only. There were no symptoms at this stage in plots where $MnSO_4$ only or $MnSO_4$ + S had been placed with the seed. A fortnight later however, symptoms appeared in these plots. The placement treatments gave less vigorous growth and poorer correction of the deficiency in oat than the foliage spray treatments of Experiment 1.

Yield Data.

Experiment 1. The statistical results of the dry weight yields of oat in lb. per plot are given in Table I.

TABLE I.

Effect of Foliage Sprays on Dry Weight Yields of Oat. (lb. per plot).

	A	B	C	D	
	No spray	0·1% foliage spray	0·25% foliage spray	0·5% foliage spray	S.D. at P = 0·01
Mean of 4 plots	2·6	16·7	18·4	21·7	6·6

D, C and B > A.

Experiment 2. In the sulphur placement experiment there were no significant differences in the yields as shown in Table II. These were low irrespective of treatment.

TABLE II.

Effect of S Placement with the Seed on the Dry Weight Yields of Oat. (lb. per plot).

	A	B	C	D	
	nil	2 cwt. S per acre	4 cwt. S per acre	8 cwt. S per acre	S.D. P = 0·05
Mean of 4 plots	2·2	2·6	2·9	2·8	1·1

Experiments 3 and 4. In Table III the results show that the placement of $MnSO_4$ with the seed has increased the fresh weight of oat, but that S alone is without effect.

TABLE III.

EFFECT OF S AND $MnSO_4$ PLACEMENT WITH THE SEED ON FRESH WEIGHT YIELDS OF OAT. (LB. PER PLOT).

		A	B	C	D	
		nil	$MnSO_4$ 96 lb. per acre	S 4 cwt. per acre	$MnSO_4$+S	S.D. P = 0·05
Mean of 4 plots	Expt. 3	21·7	25·6	21·5	26·4	3·55
Mean of 4 plots	Expt. 4	27·6	38·1	28·8	41·1	9·40

P = 0·05. D, B > A, C.

Plant Analysis.

Experiment 1. Chemical tissue tests for K, Mg, Ca, P, nitrate N and Mn were made on the mid-stem leaves, five times during the season. The mineral content of the ash was also determined. As the tissue tests are in the main in agreement with the ash analysis, the latter only will be reported in this paper. There was no consistent relation between Mn deficiency and the accumulation of nitrate N in the extracts of mid-stem leaves, as has been observed for some crops (9).

Seasonal Trends of Mineral Nutrients.

A summary of the statistical analysis of the chemical data for Experiment 1 is given in Table IV.

At the first two samplings all the sprayed plants had significantly higher Mn values in the ash than the control treatments. At the 3rd and 4th samplings graded differences are shown between the various spray treatments. At the last sampling there were no differences in Mn status of the mid-stem leaves, and at this stage "grey speck" symptoms appeared in the sprayed treatments despite very marked vigour and yield differences between sprayed and non-sprayed plants.

The interaction of other mineral nutrients with Mn is also shown in Table IV. Magnesium was significantly depressed by Mn spray treatment at the 3rd and 4th sampling times. At the 3rd sampling, P was significantly greater in the Mn treated plants.

The seasonal trends for the nutrients are given in Fig. 2. The K values which were high during the early season fell to lower levels after the 4th sampling. The Mn deficient plants tended to have higher K status than other treatments but this trend was not significant. The Ca values after an initial rise fell to about 1% CaO at the 4th sampling stage, coinciding with high K contents, and later increased when the K status fell. The Mg level in the

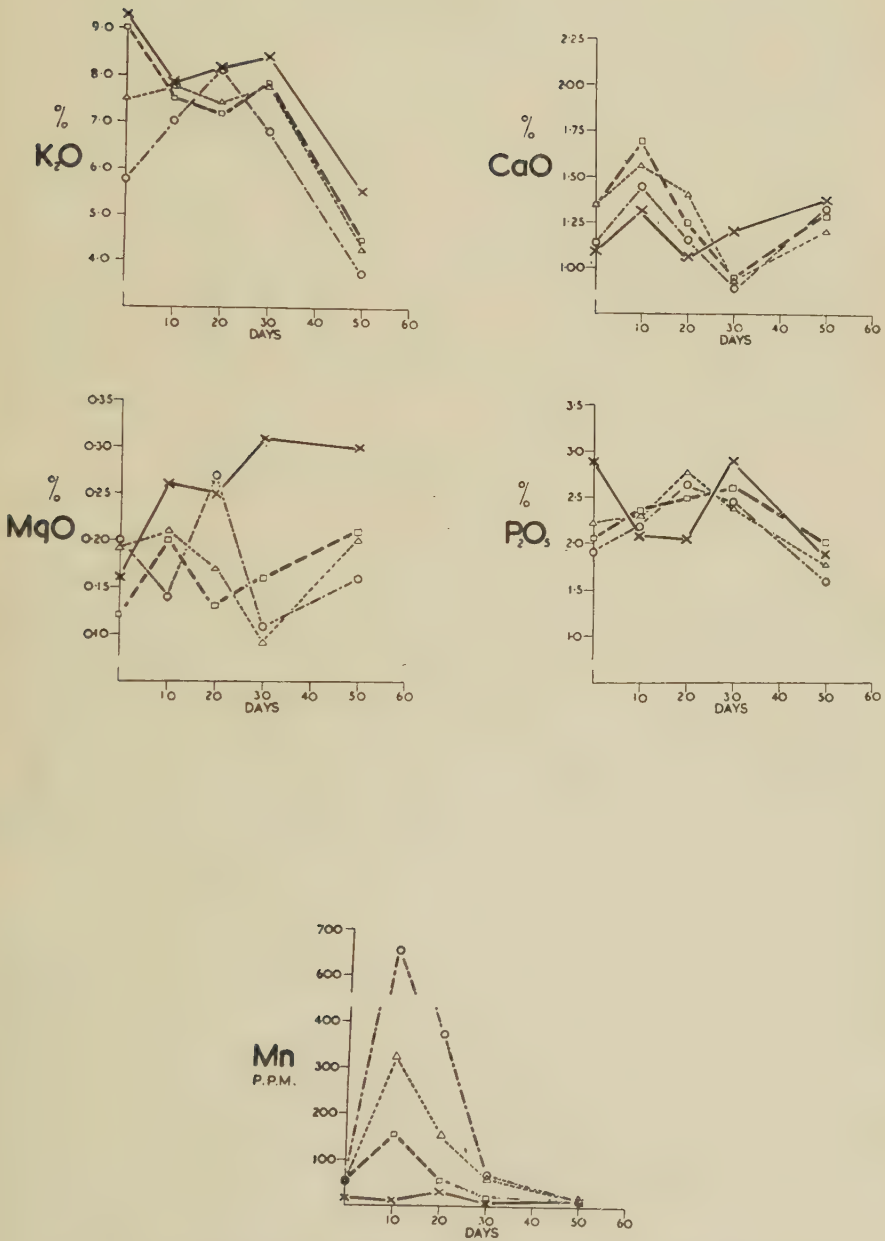


Fig. 2. The Seasonal Trends for K, Ca, Mg, P and Mn in dry matter, in relation to the following spray treatments with manganese sulphate :—

×———×	nil.
□———□	0·1%.
△- - - - - -△	0·25%.
○ — · — · ○	0·5%.

TABLE IV.

Experiment 1. Statistical Results of the Ash Analysis of the Mid-Stem Leaves of Oat ($P = 0.01$).

Seasonal Sampling	Ash Analysis.				
	Mn	K	Mg	Ca	P
9/5/49	DCB > A	N.S.	N.S.	N.S.	N.S.
19/5/49	DCB > A	N.S.	N.S.	N.S.	N.S.
30/5/49	D>C>B>A	N.S.	A > BCD	N.S.	DCB > A
9/6/49	CD > AB	N.S.	A > BCD	N.S.	N.S.
20/6/49	N.S.	N.S.	N.S.	N.S.	N.S.

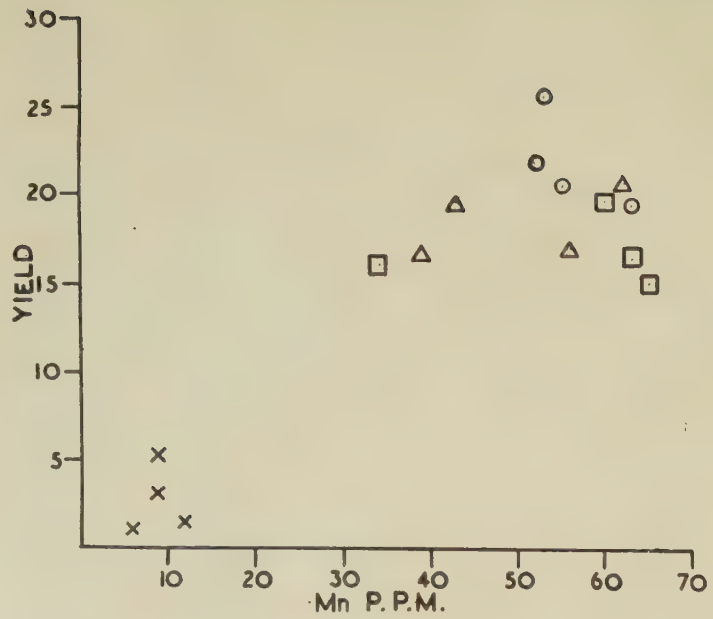
Treatments : A—nil.
B—0.1% $MnSO_4$ as foliage spray.
C—0.25% ,, ,, ,, ,,
D—0.5% ,, ,, ,, ,,

non-treated plants increased throughout the season. At the 4th sampling there was a significant decrease in the Mg levels in the Mn spray treatments. The P content of the latter treatments at the 3rd sampling were significantly lower than those given Mn. The Mn status of the 1st sampling after 2 foliage sprays, varied from 34 to 62 p.p.m., whereas control plants had between 6 and 12 p.p.m. At the second sampling after an additional foliage spray, the Mn levels were very high in the plants sprayed with 0·25 or 0·5% $MnSO_4$. These high levels were probably due to spray residues present on the leaves at this stage. At the 4th and 5th samplings, when spraying had been discontinued, the Mn levels in the mid-stem leaves fell to deficiency levels coinciding with the onset of symptoms of " grey speck " in the leaves.

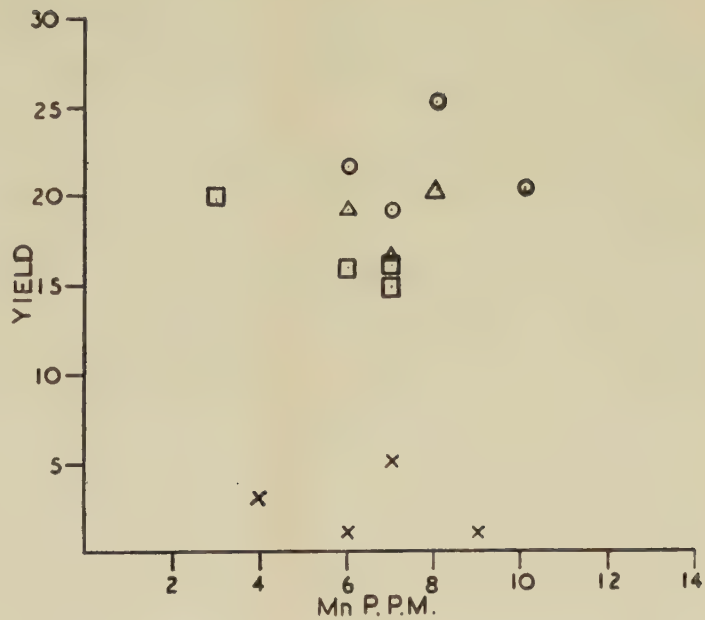
Chemical Status in Relation to Yields.

In Table IV, the statistical analyses of the Mn status of mid-stem leaves for the first two samplings show the same order of significance as for the dry weight yields of oat. At later samplings however there was little or no correlation between the Mn status and final yields. At the 5th sampling symptoms of Mn deficiency appeared in oat given spray treatments, and Mn status of mid-stem leaves were low. At this stage the Mn content of these leaves reflected the visual symptoms but not the final yields. In Fig. 3 the dry weight yields are plotted against the Mn status of mid-stem leaves at the 1st and 5th samplings respectively. At the 1st sampling an increase in Mn status was paralleled by an increase in yields but at the 5th sampling there was no correlation. At this centre, values below 40 p.p.m. in dry matter of mid-stem leaves of oat at the 1st sampling may result in a reduction in final yields. At later samplings the determination of critical values for Mn is complicated by the presence of spray residues of $MnSO_4$ on the leaves.

The results of the analysis of covariance of Mn status of the leaves and final yields are given in Table V.



A



B

Fig. 3. Relation between dry weight yields of oat in lb. per plot, and Mn status, in p.p.m. drymatter, of the mid-stem leaves.

A—1st Sampling (9th May). B—5th Sampling (20th June)

× nil.
□ 0·1% $MnSO_4$ foliage spray.
△ 0·25% " " "
○ 0·5% " " "

TABLE V.

Analysis of Covariance ("r" values) for Mn Status of Mid-Stem Leaves of Oat and Final Yields.

Sampling	Total	Treatment	Error
1st Sampling	0·87 * * *	0·97 * *	0·60
2nd Sampling	0·75 * * *	0·83 *	0·22
3rd Sampling	0·60	0·75	0·63
4th Sampling	0·41	0·76	0·17
5th Sampling	0·13	0·46	–0·68

* * * P = 0·001
* * P = 0·01
* P = 0·05

At the 1st sampling there was a significant correlation between Mn status of the mid-stem leaves and final yields based on total and treatment "r" values. At the 2nd sampling the treatment "r" value was significant at P = 0·05 level only. At later samplings the Mn status and yields were not significantly correlated.

Correlation coefficients for percentage increase in yields of Mn treated plots over control plots and Mn status of mid-stem leaves, showed similar trends to the "r" values given in Table V. At the 1st sampling the total "r" value was highly significant at 0·93 (P = 0·001). The minimum value for Mn in mid-stem leaves above which an increase in yield as a result of Mn treatment is unlikely to occur, approximates to 40 p.p.m. at this stage. Later in the season however, spray residues on the plants preclude the determination of critical Mn levels.

Discussion.

The experiments described above were designed to find the best correction for Mn deficiency in oat and to determine the relation between Mn status of the plants and final yields.

It is noteworthy that S placed with the oat seed at 2, 4 and 8 cwt. per acre did not correct the severe Mn deficiency at this centre. Manganese sulphate at 96 lb. per acre placed with the seed resulted in an increased yield but the deficiency was not fully controlled. The most effective treatments were foliage sprays at 0·1, 0·25 and 0·5% $MnSO_4$ respectively applied four times during the season making total applications of 10, 25, 50 lb. $MnSO_4$ per acre respectively. Nevertheless even in these treatments, "grey speck" symptoms appeared in the older leaves late in the season when spraying had been discontinued. At this stage chemical tissue tests for Mn showed that Mn had been translocated from mid-stem leaves to the younger leaves and grain.

A study of the Mn status of oat in relation to final yields depends on the use of appropriate methods to overcome the deficiency. The placement of S alone with the seed was ineffective as a corrective, and the inclusion of $MnSO_4$ only partially so. The foliage spray treatments resulted in the highest

yields but the determination of Mn status of these plants was complicated by spray residues on the foliage. It is unfortunate that as yet no soil method is as effective as foliage sprays in correcting Mn deficiency. Soil treatment would overcome the problem of spray residues of manganese sulphate on the leaves.

At the 1st and 2nd samplings, statistical analysis of the yield data paralleled those of the Mn status of the mid-stem leaves but at subsequent dates the correlation coefficients were not significant. Late in the season when spraying had been discontinued, visual symptoms appeared in the mid-stem leaves in the sprayed plants, and the Mn status of the leaves were as low as those of the control treatments. Thus at this stage, symptoms were in agreement with Mn content but not with final yields. This emphasises the importance of time of sampling in relating Mn status to final yields. The results show that early samplings are better, when spray residues are likely to be small, and information on response to Mn treatment of more value, as curative spray treatments can then be employed with good effect.

A minimum value for Mn in mid-stem leaves over and above which an increase in yield is unlikely to occur as a result of increasing Mn, approximated to 40 p.p.m. in dry matter at the 1st sampling. This value based on a regression of percentage increase in yield of treated over control plots and Mn status of mid-stem leaves, is only tentative as it is based on few values obtained at one centre only. Critical values for Mn at later samplings were not determined as the correlation coefficients between yields and chemical status were not significant. The minimum Mn value suggested herein for oat, 40 p.p.m. in dry matter, approximates to those given by Goodall for wheat viz., 34 p.p.m. for lower leaf blades during tillering and 36 p.p.m. in stems prior to leaf emergence (8). These values are however higher than the minimum values given by other workers for the onset of symptoms of Mn deficiency in oat viz., between 10 and 20 p.p.m. Mn in dry matter (7, 17, 20). It is clear that maximum yields occur at higher levels of Mn than those associated with deficiency symptoms. Therefore a forecast of yield response to Mn treatment can only be made by determining chemically the minimum level of Mn in the plant above which a response to Mn is unlikely to occur. Further work is necessary to establish whether such a minimum value is applicable to other Mn deficient areas and whether it is subject to seasonal change.

Summary.

1. Field experiments are described in which the efficacy of various soil treatments and foliage sprays of $MnSO_4$ for the correction of Mn deficiency in oat are compared.
2. The placement of S at 2, 4 and 8 cwt. per acre with the seed was without effect, whereas placing $MnSO_4$ at 96 lb. per acre increased the yields ($P = 0.05$). Foliage sprays of $MnSO_4$ at 0·1%, 0·25% and 0.5% respectively, applied at intervals during the season, resulted in highly significant increases in final yields of oat ($P = 0·001$).
3. Despite highly significant increases in final yields resulting from foliage spray treatments of $MnSO_4$, symptoms of Mn deficiency appeared in the sprayed plants late in the season, after spraying had been discontinued. At this stage Mn status of the mid-stem leaves was low, thus reflecting the visual symptoms but not the final yields.

4. The K, Mg, Ca, P and Mn status of the mid-stem leaves was determined at ten day intervals during the season. Magnesium was significantly depressed at the 3rd and 4th sampling, and P significantly increased at the 3rd sampling as a result of Mn foliage sprays.
5. The correlation between Mn status of oat and final yields was complicated by the presence of residues of $MnSO_4$ on leaf surfaces in the 0·25% and 0·5% spray treatments, and by the relatively small increases in yields resulting from soil treatments. Moreover, even in the spray treatments, Mn contents of mid-stem leaves were low late in the season when spraying had been discontinued.
6. Correlation coefficients between the Mn content of mid-stem leaves and final yields are highly significant for the 1st sampling when spray residues were at a minimum. At subsequent samplings however the correlation coefficients decreased and became non-significant.
7. The importance of time of sampling in relation to final yields is emphasised. At the 1st sampling, 40 p.p.m. Mn in dry matter of mid-stem leaves of oat is suggested as a tentative minimum level for this centre, above which an increase in yield as a result of Mn treatment is unlikely to occur. This critical value however, is based on the results of one experiment and needs confirmation.
8. Further work is necessary to find suitable soil methods for correcting Mn deficiency in oat so as to overcome the problem of spray residues on the plants. It will then be possible to determine, at intervals during the season, the minimum Mn values required for maximum yields at various centres deficient in manganese.

Acknowledgments.

We are grateful to Miss Olive Woodhouse and Mr. E. Catlow for assistance with establishing, recording, and sampling the oats in the field experiments. Our thanks are also due to Mr. Maud who kindly gave us permission to carry out these experiments in his extensive garden. The photographic records are the work of Mr. G. H. Jones, A.R.P.S., to whom grateful acknowledgment is made.

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SOME EFFECTS OF METALS IN EXCESS ON CROP PLANTS GROWN IN SOIL CULTURE. III. EFFECTS OF COBALT, NICKEL AND ZINC ON GROWTH, METAL AND CHLOROPHYLL CONTENTS OF TOMATO.

By D. J. D. NICHOLAS.

In Part I of this series the effects of adding Cu, Zn, Pb, Co, Ni and Mn to soil singly at 0, 5 and 10 milliequivalents (m.eq.) respectively on the growth and mineral status of tomato were investigated in a factorial pot experiment (17). It was shown that Co or Ni treatments induced iron deficiency and direct metal injury in tomato more readily than did Cu or Zn at similar m.eq. rates. The Mn or Pb treatments did not affect visibly the iron metabolism. It was also shown that total iron contents of the young leaves did not reflect iron deficiency symptoms. Fe contents of water extracts of young leaves showing iron deficiency were significantly less than those for normal leaves. Moreover chlorophyll content was correlated with the water extractable iron of young leaves.

In this paper (Part III) the effects of Co, Ni and Zn at three m.eq. rates given singly and in combination on tomato grown in soil, were studied in two factorial pot experiments. The effect of adding sodium molybdate at two rates to the highest metal combination was also investigated in an extra replicate. It is shown that water extracts of chlorotic leaves reflect the metal treatment and usually have significantly less iron than green leaves, thus confirming the data given in Part I. Contrary to Millikan's findings (11, 12, 13), Mo did not alleviate Fe deficiency induced by an excess Co, Ni and Zn.

Experimental.

Pot Experiments.

The soil used in the two factorial experiments was a sandy loam derived from Old Red Sandstone having a pH of 5·5 and was similar to that described in Part I. For these experiments the soil was however, mixed with granite chippings in the proportion of 2 of soil to 1 of chippings. The pH of the potting mixture was 6·0. In the first experiment unsterilised soil was used but in the second experiment the soil was steam sterilised to offset a fungus infection which had occurred in the glasshouse. The pots were treated with bitumen as described previously (17).

In each of the two factorial experiments there were 216 pots contained in 4 blocks, with 27 metal treatments per block and two 6″ pots per treatment. As the soil was of poor fertility, a supplement of NaH_2PO_4 (0·21 gm.) $MgSO_4 \cdot 7H_2O$ (2 gm.) and KNO_3 (1·65gm.) was given per pot containing 1·8 kilo of soil.

In Experiment 1, the soil was treated with Co, Ni and Zn, singly and in combination at 0, 1 and 2 m.eq. per pot for Co and Ni respectively and 0, 2 and 4 respectively for Zn. The 27 metal treatments were applied to the soil in solution as sulphates on 23rd May and the NPK Mg. supplement was added on 26th May. The tomato plants were transplanted into pots on 28th May when they were about a month old. In an extra series, ammonium molybdate was applied on the 23rd May to the highest metal combination at 0, 150, and 300 mg. Mo per pot.

In Experiment 2, which started in August, the rates of metal were twice those of Experiment 1, i.e. Ni and Co at 0, 2 and 4 m.eq. and Zn at 0, 4 and 8 m.eq. per pot. The metals were applied to the soil in solution as sulphates on August 1st. An extra series to test the Mo effect on iron deficiency was again included as for Experiment 1.

In the text the small figures adjacent to the metal symbols indicate the m.eq. rates applied per pot, e.g. Co_0, Ni_1, Zn_4.

Sampling Procedure.

In Experiment 1 the plants were sampled about three weeks after metal treatment. Two 3 gm. portions of young leaves were used for chlorophyll determination and extraction of Co, Ni, Zn and Fe. Chlorophyll was extracted from the leaves in an N.I.R.D. macerater using 30 ml. 85% acetone made up to 50 ml. 20 ml. of the chlorophyll in acetone was then transferred to an equal volume of ether and the percentage transmittance of the solution determined at two wavelengths 6,600 and 6,425, by means of a spectrophotometer (3,4,23). Subsequent chlorophyll extracts were then determined by means of the Spekker. The metals were extracted from the leaves with glass distilled water in a pyrex glass macerater (17). The leaves remaining were dried in beakers and the total metal content determined in the ash. The dry weights of plant "tops" and roots respectively were determined. The plant roots were washed in a special apparatus (2).

In Experiment 2, the plants were sampled two weeks after metal treatment as described in Experiment 1. Two plants were used per treatment in this experiment, so that similar leaves were taken for extracting chlorophyll and metals. In this respect more young leaf material was available than in Experiment 1 where one plant only was used because of a fungus infection.

Chemical and Ion Exchange Methods.

Water extracts of leaves prepared in the glass macerater were made to 50 ml. with glass distilled water and sub-divided into two lots of 25 ml. Co, Ni, Zn and Fe contents were determined in one 25 ml. portion after digesting with 10 ml. HNO_3 (redistilled) and 2 ml. of $HClO_4$. Digestion was continued to the "incipient dryness" stage and, after cooling, the residue was taken up in 20 ml. of glass distilled water (g.d.w.) by boiling for 15 min. The solution was then made to 25 ml. in a graduated flask with g.d.w. Appropriate aliquots

Effect of increasing Co at the highest levels of Ni and Zn on the incidence of iron deficiency in tomato.
Left $Ni_2\ Zn_4\ Co_0$ No marked symptoms.
Centre $Ni_2\ Zn_4\ Co_1$ Slight iron deficiency effects.
Right $Ni_2\ Zn_4\ Co_2$ Plant stunted, diffuse iron chlorosis in young leaves.
N.B. Increasing Ni in presence of Co and Zn at high levels gave similar effects.

Effect of increasing Zn at the highest levels of Co and Ni on the incidence of iron deficiency in tomato.
Left $Co_2\ Ni_2\ Zn_0$ Iron chlorosis in young leaves.
Centre $Co_2\ Ni_2\ Zn_2$
Right $Co_2\ Ni_2\ Zn_4$ Iron chlorosis : more in the diffuse pattern than in other treatments.

Effect of increasing Mo at the highest levels of Co, Ni, and Zn on the incidence of iron deficiency in tomato.
Left : Co_2 Ni_2 Zn_4 Mo_0. Iron deficiency in young leaves.
Centre : Co_2 Ni_2 Zn_4 Mo_1. ,, ,, ,, .. .
Right : Co_2 Ni_2 Zn_4 Mo_2. ,, ,, ,, .. ,, More diffuse pattern.

A. B. C. D.
Copper induced iron deficiency in oat.
A. 48 m.eq. Cu per pot.
B, C. 24 ,, ,, ,, ,,
D. 0 ,, ,, ,, ,,
Note chlorosis of the younger leaves and stunted roots in plants treated with copper.

were taken for chemical tests. The other 25 ml. was passed through a clearing column of glass wool (purified by boiling in redistilled nitric acid then rinsing with g.d.w.) on to a purified cation exchange column (Amberlite IR.100) (17). The metals were determined in the eluant after digesting with HNO_3 and $HClO_4$ as described above. In this way the quantity of free and bound metal in the extract was determined.

Iron was determined with dipyridyl (14,18) cobalt with nitroso-R-salt (19). nickel with dimethylglyoxime (8,19) and zinc with dithizone (7).

Results.

Visual Symptoms.

In Experiment 1, iron deficiency appeared in the Co_2 and Ni_2 treatments, a week after application of the metals. A few days later necrotic patches appeared on the young leaves (17, 21). No well defined symptoms were recorded in the plants given Zn treatment only, despite the higher m.eq. levels of application compared with Co or Ni. The symptoms recorded in Experiment 2 were similar to those observed in 1 (Plates IV and V).

Dry Weight Yields.

The statistical analysis showed that Co, Ni or Zn alone did not affect the yields of roots.

The interaction of Ni and Co, however, had a significant effect on root weights as shown in Table I.

TABLE I.

The Interaction of Co and Ni on Dryweights of Tomato Roots.

Metal applied as m.eq. per pot	Effect on dryweight—P	Metal applied as m.eq. per pot	Effect on dryweight—P
Co_0	$Ni_0 > Ni_1$ at 0·05	Ni_0	$Co_0 > Co_1$, Co_2 at 0·05
Co_1	$Ni > Ni_0$, Ni_2 at 0·05	Ni_1	$Co_1 > Co_0$ at 0·05
Co_2	N.S.	Ni_2	N.S.

Table I shows that increasing either metal in the absence of the other tends to depress the dryweights. When one metal is given at the highest rate increasing the other does not further decrease the dryweight yields. Thus the two metals depress yields independently but together do not at the highest rates exert a cumulative effect on root weights.

In the second sampling Co alone significantly depressed the root weights as shown in Table II.

TABLE II.

Experiment 2.

The Effect of Co on Dry Weights (gm.) of Tomato Roots.

Metal applied m.eq. per pot	Co_0	Co_2	Co_4
Mean of 36 values ..	0·596	0·518	0·492

	P.	S.D.
$Co_0 > Co_4$	at 0·001	0·087
$Co_0 > Co_2$	at 0·01	0·067

In the tomato tops Co or Zn treatment reduced the yields of tops in Experiment 1, three weeks after the metal treatment. In Experiment 2 there were no effects on tops harvested a fortnight after treatment.

TABLE III.

Experiment 1.

The Effect of Co and Zn Respectively on the Dry Weights (gm.) of Tomato "Tops."

Metal applied m.eq. per pot	Co_0	Co_1	Co_2	Zn_0	Zn_2	Zn_4
Mean of 36 values ..	4·21	4·60	3·48	4·26	4·54	3·48

	P.	S.D.		*P.*	S.D.
$Co_1 > Co_2$	at 0·01	0·83	$Zn_1 > Zn_4$	at 0·01	0·83
$Co_0 > Co_2$	at 0·05	0·63	$Zn_0 > Zn_4$	at 0·05	0·63

Co at 2 m.eq. and Zn at 4 m.eq. have depressed yields of tops but were without effect on the roots in the first experiment.

The addition of Mo at the two rates did not increase the dry weight yields of tops or roots at the highest rates of metal, viz. Co_2, Ni_2, Zn_4 in the first experiment. In the 2nd experiment, Mo had no effect on dry weights of the roots, but the tops at the Mo_1 level (150 mg. Mo per pot) showed an increase ($P = 0·05$) over the omit Mo treatment.

Chemical Data.

Co, Ni and Zn contents of Macerater Extracts. The metal contents of water extracts of young leaves prepared in the glass macerater are shown in Table IV and in Fig. 1 A and B.

Table IV and Fig. 1A show that the water extracts of young leaves reflect the metal treatment given to tomato. The results confirm the value of the rapid tissue test procedure for Co, Ni and Zn when present in excess in plants.

The interaction of metal treatments on Co, Zn, and Ni in the extracts is illustrated in Table V and Fig. 1B for Experiment 1.

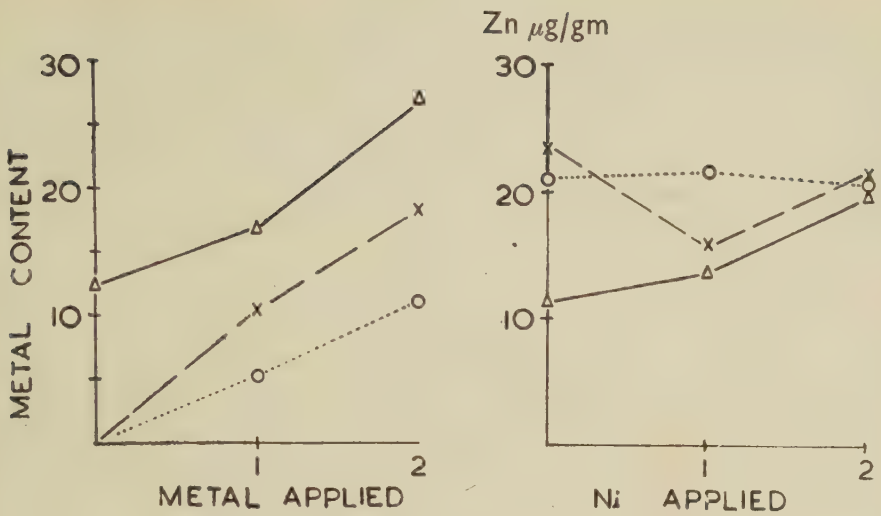

Fig. 1. *Experiment* 1.

A.—The effect of Co, Ni and Zn singly and in combination on the metal contents of water extracts of young leaves of tomato (µg. metal extracted per gm. fresh weight). Co or Ni given at 0, 1 and 2 m.eq.; Zn at 0, 2 and 4 m.eq. per pot respectively.
Δ ———— Δ Zn; X — — — — X Co; O ..... O Ni.

B.—The effect of Co and Ni on Zn content of water extracts of young leaves of tomato (µg. Zn extracted per gm. fresh weight). Ni and Co applied at 0, 1 and 2 m.eq. per pot respectively.
X — — — X Co_0; O ..... O Co_1; Δ ———— Δ Co_2.

TABLE IV.

Experiment 1.

Relation between Metal Treatment and Co, Ni and Zn Contents of Water Extracts of Young Leaves of Tomato.

Metal applied per pot	Co_0	Co_1	Co_2	Ni_0	Ni_1	Ni_2	Zn_0	Zn_2	Zn_4
Mean of 36 values µg per gm. fresh weight..	0*	10·4	17·2	0*	5·5	11·2	12·5	16·8	27·4
	$Co_2 > Co_1 > Co_0$ at *P.* 0·001			$Ni_2 > Ni_1 > Ni_0$ at *P.* 0·001			$Zn_4 > Zn_2, Zn_0$ at *P.* 0·001 S.D. 5·06		
							$Zn_2 > Zn_0$ at *P.* 0·01 S.D. 3·8		

* Not detected.

Table V shows that the Ni content of the extracts was significantly increased by Co treatment. Zn application also resulted in higher Ni values. Thus Co or Zn significantly increased Ni content of leaf homogenates.

Another effect is shown in Table VI and Fig. 1B that of Co and Ni treatment on the Zn content of the leaf extracts.

TABLE V.

Experiment 1.

The Effect of Co and Zn Respectively on Ni Content of Water Extracts of Young Leaves of Tomato.

Metal applied m.eq. per pot	Co_0	Co_1	Co_2	Zn_0	Zn_2	Zn_4
Mean of 36 values Ni as g per gm. fresh weight	7·6	8·2	9·4	7·5	8·6	8·9

	P.	S.D.		*P.*	S.D.
$Co_2 > Co_1 > Co_0$ at	0·01	0·46	Zn_4, $Zn_2 > Zn_0$ at	0·01	0·36

TABLE VI.

The Effect of Co and Ni on the Zn Content of Water Extracts of Young Leaves.

Metal applied as m.eq. per pot	Effect on Zn content—*P*	Metal applied as m.eq. per pot	Effect on Zn content—*P*
Co_0	Ni_0, $Ni_2 > Ni_1$ at 0·05	Ni_0	Co_0, $Co_1 > Co_2$ at 0·001
Co_1	N.S.	Ni_1	$Co_1 > Co_2$ at 0·01 $Co_0 > Co_2$ at 0·05
Co_2	$Ni_2 > Ni_1$, Ni_0 at 0·01	Ni_2	N.S.

Table VI and Fig. 1B show that in the absence of Ni, increasing Co significantly decreased Zn content. At Ni_2 increasing Co is without further effect on Zn. Increasing Ni at the Co_2 level results in an increase in Zn values in the extracts.

The effect of ammonium molybdate on extractable metal is shown in Table VII.

TABLE VII.

The Effect of Ammonium Molybdate at 3 Levels; 0, 150 and 300 mg. Mo per Pot on Co and Zn Content of Extracts of Tomato Leaves from the Co_2, Ni_2, Zn_4 Treatments.

Metal tested	Co			Zn		
3 levels of ammonium molybdate added to Co_2, Ni_2, Zn_4 treatment	Mo_0	Mo_1	Mo_2	Mo_0	Mo_1	Mo_2
Metal content as μg per gm. fresh weight	17·5	28·6	22·9	33·5	22·1	19·0

	P.	S.D.		*P.*	S.D.
$Mo_1 > Mo_0$ at	0·05	7·3	$Mo_0 > Mo_1$, Mo_2 at	0·01	9·8

Mo_1 increased the extractable Co from young leaves and the two Mo levels decreased Zn status of the extracts. Mo had no effect on Ni content of the leaves.

After passage of the water extracts through Amberlite IR.100 columns, the following levels of metal were recovered in the eluant, 95% Ni, 60% Co and 50% Zn. These values indicate the extent of binding of the metals in the homogenates. The incidence of iron chlorosis and metal injury is in the order Ni > Co > Zn and is paralleled by the degree of fixation of the metals in the water extracts of the young leaves.

TABLE VIII.

EXPERIMENT 2.

THE EFFECT OF NI AND CO RESPECTIVELY ON FE EXTRACTED FROM YOUNG LEAVES.

Metal applied as m.eq. per pot	Ni_0	Ni_4	Ni_8	Co_0	Co_2	Co_4
Fe as μg per gm. fresh weight. Mean of 36 values	3·8	2·7	3·2	3·9	2·9	3·0

	P.	S.D.		*P.*	S.D.
$Ni_0 > Ni_4$	at 0·001	0·82	$Co_0 > Co_2$, Co_4	at 0·001	0·82
$Ni_0 > Ni_8$	at 0·01	0·60			

The interactions of Co, Ni and Zn on Fe in leaf homogenates in Experiments 1 and 2 are illustrated in Table IX and Fig. 2.

TABLE IX.

THE INTERACTION OF CO AND NI ON FE EXTRACTED FROM YOUNG LEAVES.

EXPERIMENT 1.

Metal applied as m.eq. per pot	Effect on Fe extracted—*P*	Metal applied as m.eq. per pot	Effect on Fe extracted—*P*
Co_0	$Ni_0 > Ni$ at 0·001 $Ni_0 > Ni_2$ at 0·05	Ni_0	$Co_1 > Co_2$ at 0·001 $Co_0 > Co_2$ at 0·05
Co_1	$Ni_0 > Ni_1$, Ni_2 at 0·001	Ni_1	$Co_2 > Co_1$, Co_0 at 0·001
Co_2	$Ni_1 > Ni_2$ at 0·01 $Ni_1 > Ni_0$ at 0·01	Ni_2	N.S.

EXPERIMENT 2.

Co_0	$Ni_0 > Ni_2$, Ni_4 at 0·01 $Ni_4 > Ni_2$ at 0·05	Ni_0	$Co_0 > Co_2$, Co_4 at 0·01
Co_2	$Ni_0 > Ni_1$ at 0·05	Ni_2	N.S.
Co_4	N.S.	Ni_4	$Co_0 > Co_2$ at 0·05

Iron Content of Macerater Extracts.

In Experiment 1, increasing Ni significantly reduced the iron content of water extracts of young leaves but Co or Zn treatments were without effect. As the tip chlorotic leaves were used for chlorophyll determination, older leaves, which were not showing Fe deficiency were used for the extraction of the metals in Experiment 1. This may account for the fact that Co or Zn did not affect the extractable iron. In the second experiment, two plants were available per treatment, so that young leaves were taken for the extraction of iron. Ni and Co were also given at twice the levels in Experiment 1 and both metals significantly reduced the iron content of the extracts as shown in Table VIII and Fig. 2.

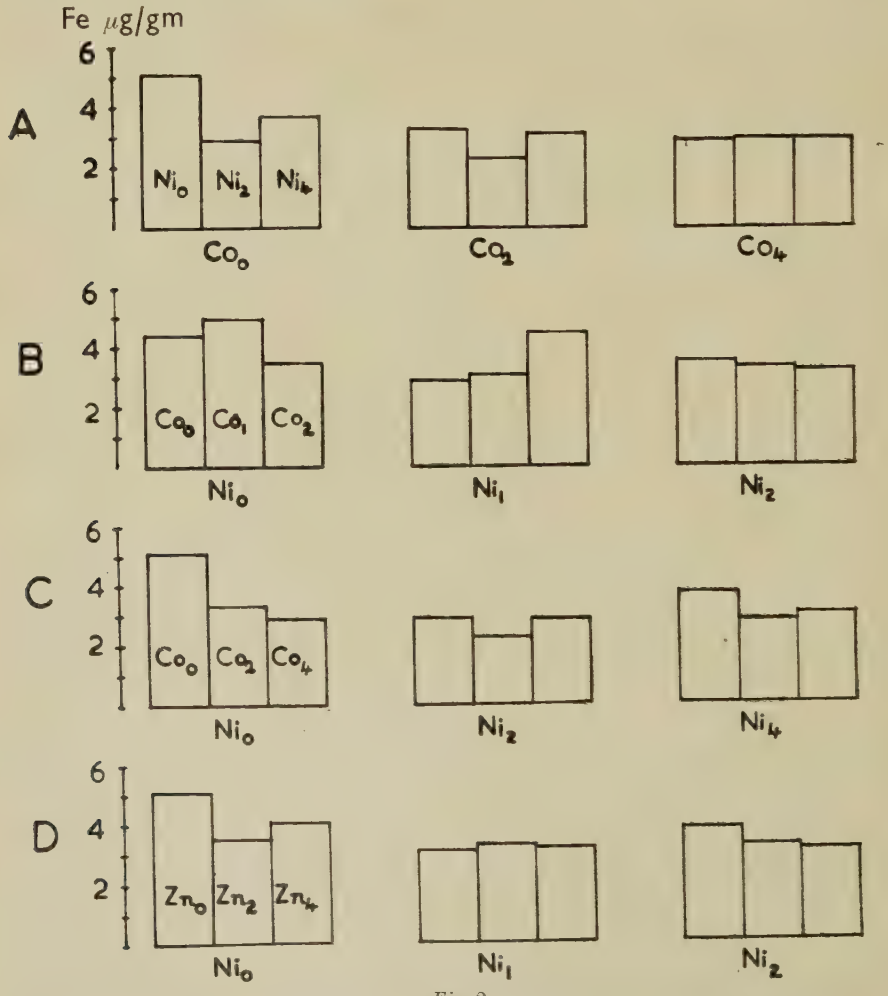

Fig. 2.

Iron content of water extracts of young leaves (as μg. extracted per gm. fresh weight).

A.—2nd experiment. The effect of Co and Ni treatment on Fe status.
B.—1st experiment. As for A.
C.—2nd experiment. As for A.
D.—1st experiment. The effect of Zn and Ni on Fe status.

Increasing Ni at Co_0 depressed the Fe content in the extracts, so did increasing Co at the Ni_0 level except for Ni_0 Co_2 in the Experiment 1 which was unchanged. An increase in Co at the high Ni level or vice versa has little effect on reducing Fe further. The interaction of Ni and Zn on Fe content showed a similar effect to those given for Ni and Co.

In the extra replicate (first experiment) Mo treatment increased the extractable Fe at the two Mo rates ($P = 0.05$ only). In the second experiment Mo had no effect on extractable Fe.

The total Fe contents of the leaves were not significantly changed by metal treatment.

The extractable iron was expressed as a percentage of the total Fe (log_{10}) and this ratio was significantly reduced for Ni and Co treatment as shown in Table X and Fig. 3.

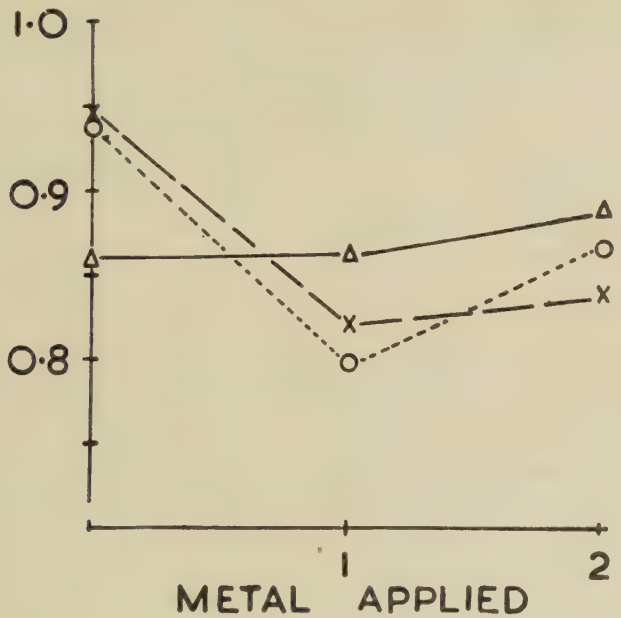

Fig. 3. *Experiment* 2.

The effect of metal treatment on Fe in water extracts of fresh leaves as percentage of the total iron (log_{10}).

Δ ———— Δ Zn ; X — — — — X Co ; O ..... O Ni.

Co or Ni applied at 0, 1 and 2 m.eq. per pot respectively.

Zn applied at 0, 2 and 4 m.eq.

TABLE X.

Experiment 2.

The Effect of Co and Ni on Fe Extracted as Percentage of the Total Fe (log_{10}).

Metal treatment m.eq. per pot	Ni_0	Ni_2	Ni_4	Co_0	Co_2	Co_4
	0·94	0·80	0·88	0·95	0·83	0·84

	P.	S.D.		*P.*	S.D.
$Ni_0 > Ni_4$ at	0·001	0·116	$Co_0 > Co_2$ at	0·001	0·116
$Ni_4 > Ni_2$ at	0·05	0·067	$Co_0 > Co_4$ at	0·01	0·089

The results in Table X and Fig. 5 confirm that the extractable iron as a percentage of the total iron in the leaves, was significantly decreased with application of Co or Ni and this trend paralleled the onset of iron chlorosis. Zn treatment however was without effect on this value and iron chlorosis was not observed in the plants given zinc. The Mo treatment had no significant effect on the iron ratio.

Chlorophyll Content. In both experiments Co, Ni and Zn treatment respectively significantly reduced chlorophyll content as shown in Table XI and Fig. 4.

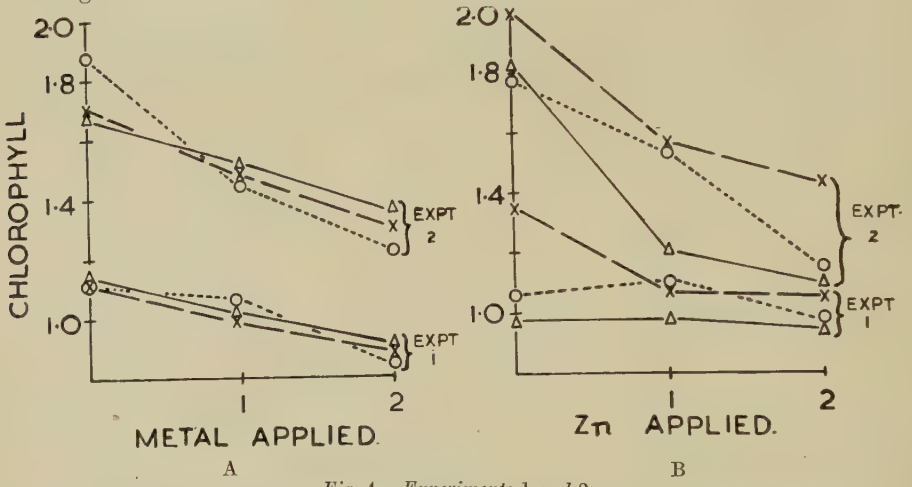

Fig. 4. *Experiments* 1 *and* 2.

A.—The effect of metal treatment on chlorophyll content of young leaves (mg. extracted per gm. fresh weight).

Δ ——————— Δ Zn ; X — — — — X Co ; O ..... O Ni.

B.—The effect of Zn and Co (Experiment 1) and Zn and Ni (Experiment 2) on chlorophyll contents of young leaves (mg. extracted per gm. fresh weight).

Experiment 1.

X — — — X Co_0.
O ..... O Co_1.
Δ ——— Δ Co_2.

Experiment 2.

X — — — X Ni_0.
O ..... O Ni_2.
Δ ——— Δ Ni_4.

TABLE XI.

The Effect of Ni, Co and Zn on Chlorophyll Content of Young Leaves.

Experiment 1.	*P*	Experiment 2.	*P*
Ni_0, $Ni_1 > Ni_2$	at 0·001	$Ni_0 > Ni_2 > Ni_4$	at 0·001
$Co_0 > Co_2$ $Co_1 > Co_2$	at 0·001 at 0·01	$Co_0 > Co_2 > Co_4$	at 0·001
$Zn_0 > Zn_1 > Zn_2$	at 0·001	$Zn_0 > Zn_4$, Zn_8 $Zn_4 > Zn_8$	at 0·001 at 0·01

The reduction in chlorophyll content by metal application is closely paralleled by an increase in the metal content of leaf homogenates. The results of the analysis of covariance ('*r*' values) for Co, Ni, and Zn status of macerater extracts and chlorophyll content in Experiment 1 ($n_2 = 17$) were as follows :—

	('*r*' values)	*P*
Co ..	–0·38	0·1
Ni ..	–0·68**	0·01
Zn ..	–0·10	0·1

The Ni content was negatively correlated with the chlorophyll content ($P = 0·01$) whereas Co and Zn levels were negatively correlated at the 0·1 level only. The linear regression terms were calculated for the three metals in relation to chlorophyll, and the regression lines are illustrated in Fig. 5. For Ni and chlorophyll the linear regression is significant at ($P = 0·01$). The regressions for Co or Zn content and chlorophyll, although falling short of significance, are included as trends in Fig. 5.

The interactions of Co and Zn (first experiment), Ni and Zn (second experiment) on chlorophyll are shown in Table XII.

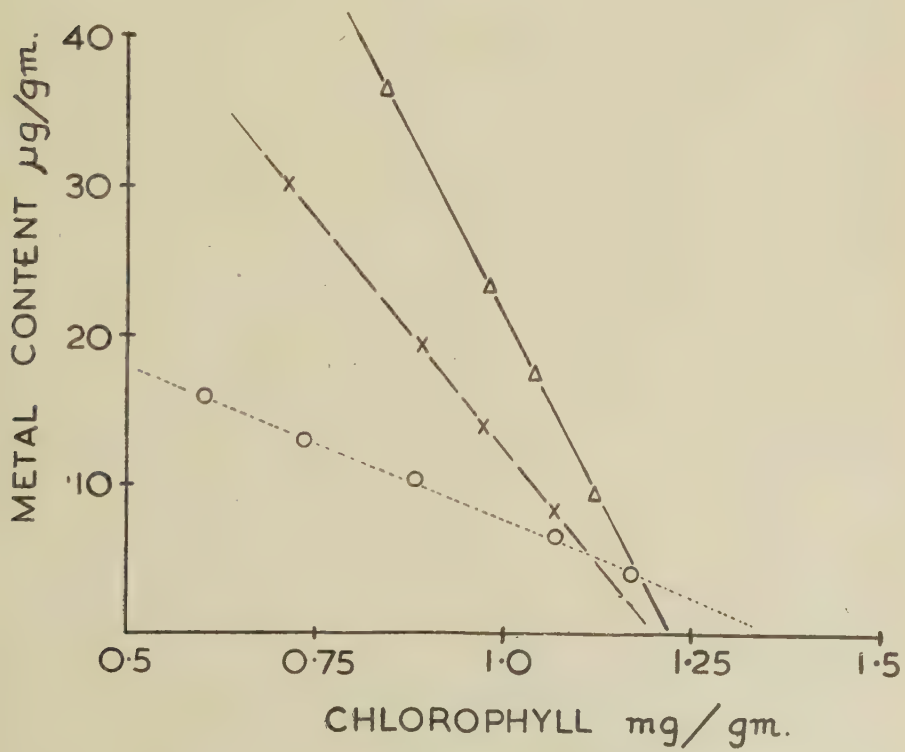

Fig. 5. Experiment 1.
Linear regression lines for metal and chlorophyll contents of extracts of young leaves.
O ..... O Ni at $P = 0·01$.
X —— —— —— X Co at $P = 0·1$.
Δ ———————— Δ Zn at $P = 0·1$.

TABLE XII.

THE INTERACTION OF METALS ON THE CHLOROPHYLL CONTENT OF YOUNG LEAVES.

EXPERIMENT 1.

Metal applied as m.eq. per pot	Effect on chlorophyll content	*P*	Metal applied as m.eq. per pot	Effect on chlorophyll content	*P*
Zn_0	$Co_0 > Co_1$, Co_2	at 0·001	Zn_0	$Zn_0 > Zn_2 > Zn_4$	at 0·001
Zn_2	$Co_1 > Co_2$ $Co_0 > Co_2$	at 0·001 at 0·01	Zn_4	$Zn_0 > Zn_2 > Zn_4$	at 0·001
Zn_4	N.S.		Zn_8	$Zn_0 > Zn_4$ $Zn_0 > Zn_2$	at 0·001 at 0·01

EXPERIMENT 2.

Zn_0	$Ni_0 > Ni_2$, Ni_4 at 0·001 $Ni_2 > Ni_4$ at 0·05	Ni_0	$Zn_0 > Zn_4$, Zn_8	at 0·01
Zn_4	Ni_0, $Ni_2 > Ni_4$ at 0·001 $Ni_0 > Ni_2$ at 0·01	Ni_2	Zn_0, $Zn_4 > Zn_8$	at 0·001
Zn_8	$Ni_0 > Ni_2$, Ni_4 at 0·001	Ni_4	$Zn_0 > Zn_4$, Zn_8	at 0·001

Increasing Zn at the lowest Co level and increasing Co at the lowest Zn level reduced chlorophyll. At Co_2 an increase in Zn further decreased the pigment. At Zn_4, however, increasing Co did not decrease chlorophyll further. Thus Zn appears to have had a significant effect on reducing total chlorophyll. Visual symptoms however, did not reflect this marked Zn effect on chlorophyll.

In Experiment 2, an increase in Ni or Zn at any level of the other significantly reduced the chlorophyll content of the young leaves and this was in accord with the visual symptoms observed.

The application of Mo did not increase chlorophyll production and had no effect on chlorosis in the plants receiving high dosages of Co, Ni and Zn.

The ratio of chlorophylls A/B was determined by means of a simple wavelength spectrometer and was found to vary from 2·85 to 3·26. There appeared to be no significant change in the ratio between normal green leaves and those showing iron deficiency, although the total chlorophyll was markedly reduced.

Measurements made for carotene and xanthophyll in normal and iron chlorotic leaves indicate that both pigments were significantly reduced in iron deficient leaves.

Discussion.

The main objects of these factorial experiments on metal-induced iron deficiency in crop plants grown in soils were : (a) to determine the applicability of rapid chemical tests for heavy metals present in excess in fresh plant material ; (b) to determine iron fractions in the tissues which would reflect iron chlorosis in plants ; (c) to study some of the biochemical aspects of metal-induced iron deficiency, e.g. effect on plant pigments ; (d) to find whether the addition of Mo would alleviate symptoms of Fe deficiency induced by metal injury.

In two large factorial experiments described in this paper, the effects of soil applications of Co, Ni and Zn on tomato var. Market King, were studied. The metals were given singly and in combination at 3 m.eq. rates making a total of 27 metal treatments. These three metals were chosen as previous experiments (17) had shown that Co or Ni readily induced Fe deficiency and that Zn was less toxic at similar m.eq. levels.

Water extracts of young leaves prepared in a glass macerater, reflected the Co, Ni and Zn treatments given. Rapid chemical tests can therefore be used instead of the time-consuming ash analysis methods, for determining quantitatively these and other metals (16, 17) when present in excess in plants. Moreover, the rapid tests are useful as a number of metals when present in excess either singly or together result in iron deficiency symptoms. It is only by the use of chemical methods that the metals involved can be satisfactorily identified and determined quantitatively.

The nature of the metals present in the water homogenates of leaves is of interest. When untreated extracts of leaves from plants treated with the three metals respectively were percolated through cation-exchange columns, 98% Ni, 60% Co, and 50% Zn were recovered in the eluant. The latter was oxidised with acids so as to determine its total metal content. The extent of metal binding in the extracts is paralleled by the severity of iron deficiency. These results also explain the reason for the non-detection of Ni in water extracts of leaves, reported in the previous paper (17) as the extracts were not treated with oxidising acids to free the bound metal.

The interactions of one metal treatment with others were reflected in the water extracts of young leaves and these can only be satisfactorily established in factorial experiments such as those described above. These effects include those of Co and Zn treatment which increased the Ni status and the application of Co which decreased the Zn content of the plants.

It is well known that the total iron content of plants is seldom related to symptoms of Fe deficiency induced by excess lime or heavy metals (1, 9, 10, 16, 17, 18, 20). In this paper further evidence is given that the water extractable iron is correlated with iron deficiency symptoms, thus confirming last year's results (17). Co or Ni readily induced Fe deficiency and the extractable iron followed the severity of the symptoms closely. Zn at similar m.eq. applications, had no significant effect on this iron fraction and symptoms of Fe deficiency were not well developed. The extractable iron expressed as a percentage of the total Fe in the plant varied from 15 in green leaves to 7 in chlorotic leaves. Thus the Fe in the water extracts of young leaves may be a measure of the " active " fraction. The " active " Fe values are small in relation to the total values in the ash and at these levels are probably involved in vital enzymic processes in relation to chlorophyll production. Work is in

progress to determine the nature of the iron fractions present in the water homogenates of green and chlorotic leaves.

The total chlorophyll was significantly reduced by metal application, thus reflecting iron chlorosis. Correlation and regression coefficients for Ni content of homogenates and total chlorophyll in young leaves were significant and those for Co or Zn were just short at ($P = 0{\cdot}1$). The correlation between chlorophyll and extractable iron was less clear. In plants given Ni and Co treatments, a decrease in "active" iron paralleled a similar depression in chlorophyll, but for Zn treatment, there was no clear relation. More work is therefore required to elucidate the relation between pigment production and extractable iron. There appeared to be no significant changes between the ratios of chlorophylls A/B with treatment; these varied from 2·75 to 3·26. Xanthophylls and carotenes were consistently reduced in young leaves as iron deficiency became more severe.

Millikan has shown that Mo counteracts Fe deficiency in flax induced by excessive amounts of heavy metals in water culture (11, 12, 13). In sand culture Hewitt found that Mo aggravated the effects produced by toxic amounts of Mn^{++}, Cr^{+++}, Zn^{++} and Cu^{++} in sugar beet grown in sand culture (5, 6). Both base their conclusions in the main on visual symptoms and dryweight yields. Millikan did not study the iron status of flax. Hewitt failed to find an iron fraction which reflected iron chlorosis and has not determined metal status of the plants. Thus it is difficult to make any valid comparisons between the results of the two workers. Warington (22) however, has examined the mineral status of soybean, flax and oat given various levels of Mn, Mo and V in water culture. In soybean and flax, high Mn levels induced iron deficiency; the addition of Mo did not offset the symptoms, but V at low levels counteracted some of the injurious effects. The beneficial effects of V were reflected by higher Fe values in the shoots. The total Fe levels (as μg per gm. dry matter), varied from 490 in normal to 60 in iron deficient shoots. These values, although reflecting iron chlorosis, are however higher than those usually found in field specimens (15). In oat contrary to soybean and flax, the total iron increased with Mn treatment (22).

No information is available on the effect of Mo on heavy metals in soils. In view of the conflicting evidence reported above and the economic importance of overcoming metal injury in areas adjacent to mine workings or agricultural land treated with certain types of industrial effluent, the effect of Mo was tried in an extra series. The results show that ammonium molybdate given at two rates to tomato did not alleviate symptoms of iron chlorosis induced by Co, Ni and Zn. The treatment is unlikely to be of value as a corrective for metal injury in tomato in the soils examined.

Summary.

(1) The effects of treating tomato, var. Market King, grown in soil with various levels of Co, Ni and Zn were examined in two factorial pot culture experiments. The three metals were given singly and in combination at three milliequivalent levels, making a total of 27 metal treatments.

(2) Co or Ni at lower m.eq. levels proved more toxic to tomato than Zn, inducing iron deficiency symptoms and direct metal injury effects.

Co reduced yields of roots and Co or Zn depressed those of "tops" in the second experiment.

(3) Co, Ni and Zn present in water extracts of young leaves prepared in glass macerators reflected the metal treatments given.

(4) The effects of two of the metals on the uptake of the other were studied by chemical tissue tests.

(5) The total iron in the ash of young leaves was not significantly changed by metal treatment and did not reflect iron deficiency symptoms. The iron present in the water extracts of young leaves usually paralleled the intensity of chlorosis, especially in the Co and Ni treated plants. As chlorosis became more marked the percentage of soluble iron to total iron was significantly decreased in the leaves.

(6) Co, Ni and Zn treatment depressed the total chlorophyll content of the young leaves but the ratio of chlorophyll A to chlorophyll B which varied from 2·75 to 3·26 was not significantly changed. Chlorophyll content was significantly correlated with metal content of the water extracts of leaves. A linear regression line fits the data for increased Ni content resulting in decreased chlorophyll status. Xanthophyll and carotene contents were also substantially reduced in chlorotic leaves.

(6) There is no clear relation between Fe content of water extracts and total chlorophyll so that further work is required to investigate this point further.

(7) Mo did not overcome the injurious effects of Co, Ni and Zn on plants, as determined by visual and chemical methods.

Acknowledgments.

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SOME EFFECTS OF METALS IN EXCESS ON CROP PLANTS GROWN IN SOIL CULTURE. IV. EFFECTS OF COPPER ON MINERAL STATUS OF CROP PLANTS.

By W. A. FORSTER.

This paper describes the results of chemical analyses made on tomato and oat harvested from the 1949 and 1950 experiments, some results of which have been described previously, and also reports some effects of copper on oat, rye, barley, sugar beet and kale grown in soils in 6″ bitumen-painted pots in the glasshouse (1).

Experimental.

1949 and 1950 Experiments.

Experiment A. Mid-stem leaves of tomato (var. Stonor's M.P.) were harvested when the plants were two months old, after they had received several applications of copper sulphate solution totalling 563 and 1,127 m.eq. Cu per pot, respectively. Control plants in this experiment received no Cu.

Experiment D. Oat plants (var. S 84) which had been treated with copper sulphate solution at 0, 17·5, 35 and 70 m.eq. Cu per pot, respectively, were sampled three times at five-day intervals. At the 1st and 2nd Samplings, plants from four pots were bulked. At the 3rd Sampling, in order to obtain an estimate of sampling error, four pots receiving the same treatment were combined in pairs, giving two duplicate series A and B. Each series was harvested by a different team of workers. The tops from series A and B from pots receiving the same treatment were combined, the roots (A and B) were kept separate.

1951 Experiments.

Experiment E. Oat (var. S 84) was grown in a factorial experiment containing 192 pots. The soil used (pH 5·9) was prepared by mixing a sandy loam pH 4·7 with granite chippings in the ratio 3:2. The mixture was not sterilised. Copper sulphate solution was applied before planting at rates 0, 12·5, 25 and 50 m.eq. Cu per pot. In addition, 16 pots of sterilised soil mixture were sown with oat. In each block there were twelve pots for each of the following treatments 0, 12·5, 25 and 50 m.eq. Cu per pot.

Experiment F. Rye (var. Giant) and barley (var. Plumage Archer) were grown in a factorial experiment of split plot design, in a soil similar to that

used in the previous experiment. There were 96 pots for each crop. Copper sulphate solution was applied before planting at 0, 5, 10, 20 m.eq. Cu per pot respectively.

Experiment G. Sugar beet (var. Klein Wanzleben) and kale (var. Borecole) were grown in a factorial experiment similar to that used for rye and barley. A soil derived from New Red Sandstone (pH 6·4) was used. Copper was applied before planting at the following rates 0, 10, 20, 40 m.eq. Cu per pot.

Results.

1949 and 1950 Experiments.

The symbols Cu_0, Cu_1, Cu_2, etc., indicate the rates of metal application. The total milli-equivalent values corresponding to these rates are given in Tables I, III and VI.

Experiment A, tomato.

TABLE I.

Some Effects of Copper on Tomato.

	Mean values averaged over six blocks, at rates of copper			L.S.D. at P		Order
	Cu_0 0 m.eq.	Cu_1 563 m.eq.	Cu_2 1127 m.eq.			
Yield of fruit	17·78	6·27	5·15	0·91	0·05	$Cu_1 > Cu_2$
				1·87	0·001	$Cu_0 > Cu_1, Cu_2$
K_2O in mid-stem leaves ..	2·76	3·03	3·41	0·24	0·05	$Cu_0 < Cu_1$
				0·35	0·01	$Cu_1 < Cu_2$
				0·50	0·001	$Cu_0 < Cu_2$
MgO in mid-stem leaves ..	1·52	1·86	1·85	0·30	0·05	$Cu_0 < Cu_1, Cu_2$
$\log_{10}$ Cu in mid-stem leaves ..	1·73	2·35	2·84	0·42	0·01	$Cu_1 < Cu_2$
				0·61	0·001	$Cu_0 < Cu_1, Cu_2$

CaO, P_2O_5, Fe and Mn were not significantly affected.
K_2O, CaO, MgO, P_2O_5 are expressed as percentage; Fe, Cu, Mn as μg per gm in dry material.
Yield of fruit in lbs. m.eq. = milli-equivalents.

Table I shows that both rates of copper significantly decreased the yield of fruit, and increased the K and Mg contents of mid-stem leaves. The copper content varied greatly, thus at 563 and 1,127 m.eq. the values ranged from 94 to 448, and 293 to 1,448 μg Cu per gm. dry matter, respectively. The leaves of plants not given Cu contained 4–6 μg Cu per gm. dry matter.

TABLE II.

THE RESULTS OF ANALYSIS OF COVARIANCE ("r" VALUES) FOR MINERAL STATUS OF TOMATO, MID-STEM LEAVES.

Factors	"r" values
Cu, Fe	—0·234 N.S.
„ K	0·704 * *
„ Ca	—0·247 N.S.
Mn, Fe	0·430 N.S.
„ P	0·597 * *
K, Ca	—0·235 N.S.
„ P	0·405 N.S.

$n_2 = 16.$

Symbol.	*P.*
N.S.	>0·05
* *	0·01

Correlation coefficients for a number of the mineral nutrients present in mid-stem leaves were determined, and are given in Table II. The correlations between (1) Cu and K, (2) Mn and P, were positive at $P = 0{\cdot}01$. It is interesting to note that Cu and Fe were not significantly correlated.

Table III shows that Cu_3 significantly reduced the yield of "tops" at the three samplings. The application of Cu significantly increased the Cu content and (at the second sampling only) decreased the K content; the levels of Ca, Mg, P, Mn and Fe were unaffected. The Ca contents at each sampling were similar, irrespective of treatment.

Correlation coefficients, given in Table IV, were determined for a number of factors relating to oat tops.

The most significant correlations, which were positive, were for (1) Fe and Mn, (2) P and K. The 'r' values for Cu and Fe in tops were significant at $P = 0{\cdot}05$ only.

Table V shows that the tops of plants given 35 m.eq. Cu contained twice as much Cu as plants given 17·5 m.eq. Increasing the rate of copper from 35 to 70 m.eq. however, resulted in a 3- to 5-fold increase in Cu content.

As indicated in Table VI, the yield of roots was significantly decreased at the 2nd Sampling only. K and P were significantly lower and Ca higher in plants given Cu. All three rates of Cu affected the Cu, K and P status, but the Ca content was affected by the highest rate of Cu only. At the 1st Sampling only, the seed and hypocotyl were included in the "roots," this resulting in the apparently higher yield as compared with the 2nd and 3rd Samplings. Ca, P and K were all higher in series A than in series B at the 3rd Sampling. In series A the root washing may have been more prolonged than in series B which would probably account for these differences (see Appendix). Statistical results show that the differences in P and K status respectively of the roots from series A and B were not affected by Cu treatment.

TABLE III.

SOME EFFECTS OF COPPER ON OAT "TOPS."

	Mean values averaged over four blocks, at rates of copper				L.S.D. at *P*		Order
	Cu_0 0 m.eq.	Cu_1 17·5 m.eq.	Cu_2 35 m.eq.	Cu_3 70 m.eq.			
Yield of tops, 1st Sampling	22·23	23·38	23·51	19·39	2·06	0·01	$Cu_0 > Cu_3$
					3·03	0·001	Cu_1, $Cu_2 > Cu_3$
,, 2nd Sampling	25·53	26·31	27·34	20·56	3·94	0·05	Cu_0, Cu_1, $Cu_2 > Cu_3$
,, 3rd Sampling	32·31	34·05	33·16	25·41	6·59	0·01	Cu_0, Cu_1, $Cu_2 > Cu_3$
$\log_{10}$ Cu, tops, 1st Sampling	0·86	1·36	1·64	2·09	0·26	0·01	$Cu_1 < Cu_2$
					0·38	0·001	$Cu_0 < Cu_1$, $Cu_2 < Cu_3$
$\log_{10}$ Cu, tops, 3rd Sampling	0·32	1·01	1·36	2·05	0·30	0·05	$Cu_1 < Cu_2$
					0·64	0·001	$Cu_0 < Cu_1$, $Cu_2 < Cu_3$
K_2O tops, 2nd Sampling	4·22	4·26	4·33	3·88	0·30	0·05	Cu_0, Cu_1, $Cu_2 > Cu_3$

The following were not significantly affected :—

K_2O, CaO, P_2O_5, Fe, Mn—1st Sampling.
CaO, MgO, P_2O_5, Fe, Mn—2nd Sampling.
K_2O, CaO, P_2O_5, Fe, Mn—3rd Sampling.

TABLE IV.

Analysis of Covariance ("r" Values) for Data Relating to Oat Tops.

Factors	Sampling		
	1st	2nd	3rd
Cu content, yield	—0·410 N.S.	—0·557 +	—0·607 *
Cu, Fe	—	0·505 +	0·555 +
,, Mn	0·227 N.S.	0·468 N.S.	0·356 N.S.
,, K	—0·370 N.S.	—0·533 +	—0·313 N.S.
,, P	—0·598 *	—0·413 N.S.	—0·169 N.S.
Fe, Mn	—	0·403 N.S.	0·769 ***
,, K	—	0·134 N.S.	0·155 N.S.
,, P	—	—0·045 N.S.	0·245 N.S.
,, Mg	—	0·107 N.S.	—
Mn, K	—0·520 +	—0·595 *	—0·041 N.S.
,, P	—0·246 N.S.	—0·038 N.S.	0·589 *
K, P		0·896 ***	

Symbol.	*P.*
N.S.	>0·05
+	0·05
*	0·02
**	0·01
***	0·001

The correlation coefficient for P and K determined on combined data of the three samplings ($n_2 = 62$). In all other comparisons $n_2 = 14$.

TABLE V.

The Effect of Cu Application on the Cu Content of Oat Tops.

μg. Cu·gm. dry matter.

M.eq. Cu applied per pot	0	17·5	35	70
1st Sampling ..	7·3	23·0	44·3	125·8
2nd Sampling ..	5·2	11·8	24·9	142·7
3rd Sampling ..	2·1	10·6	24·1	125·4

TABLE VI.

Some Effects of Copper on Oat "Roots."

		Mean values averaged over four blocks, at rates of copper				L.S.D. at P		Order
		Cu_0 0 m.eq.	Cu_1 17·5 m.eq.	Cu_2 35 m.eq.	Cu_3 70 m.eq.			
Yield of roots,	1st Sampling	14·19	12·93	13·16	11·62			not significant
,,	2nd Sampling	10·05	10·21	9·25	7·02	2·25	0·05	Cu, $Cu_1 > Cu_3$
,,	3rd Sampling	10·30	11·01	9·28	8·18			not significant
$\log_{10}$ Cu, roots,	3rd Sampling "A" . .	2·09	2·59	2·89	3·42	0·26	0·001	$Cu_0 < Cu_1 < Cu_2 < Cu_3$
K_2O, roots,	1st Sampling	2·19	1·87	1·88	2·05	0·24	0·05	$Cu_0 > Cu_1$, Cu_2
,,	2nd Sampling	1·79	1·56	1·48	1·25	0·24	0·05	$Cu_0 > Cu_2$; $Cu_1 > Cu_3$
						0·35	0·01	$Cu_0 > Cu_3$
,,	3rd Sampling "A" . .	1·55	1·31	1·31	1·00			not significant
,,	,, "B" . .	1·46	1·23	1·11	0·92	0·25	0·05	$Cu_0 > Cu_2$; $Cu_1 > Cu_3$
						0·36	0·01	$Cu_0 > Cu_3$
CaO, roots,	1st Sampling	0·738	0·747	0·781	0·864	0·066	0·05	$Cu_2 < Cu_3$
						0·095	0·01	Cu_0, $Cu_1 < Cu_3$
,,	2nd Sampling	0·574	0·564	0·587	0·666	0·059	0·01	Cu_0, Cu_1, $Cu_2 < Cu_3$
,,	3rd Sampling "A" . .	0·602	0·601	0·637	0·734	0·087	0·01	Cu_0, Cu_1, $Cu_2 < Cu_3$
,,	,, "B" . .	0·551	0·526	0·562	0·679	0·096	0·05	Cu_0, Cu_1, $Cu_2 < Cu_3$
P_2O_5, roots,	1st Sampling	0·519	0·462	0·472	0·515	0·036	0·01	$Cu_0 > Cu_1$, $Cu_2 < Cu_3$
,,	2nd Sampling	0·535	0·468	0·462	0·439	0·023	0·05	Cu_1, $Cu_2 > Cu_3$
						0·049	0·001	$Cu_0 > Cu_1$, Cu_2, Cu_3
,,	3rd Sampling "A" . .	0·583	0·450	0·468	0·443	0·071	0·05	$Cu_0 > Cu_1$, Cu_2, Cu_3
,,	,, "B" . .	0·462	0·433	0·429	0·386			not significant

K_2O, CaO, MgO, P_2O_5 are expressed as percentage; Fe, Cu, Mn as μg per gm. in dry material.
Yield data in gm. m.eq. = milli-equivalents.

TABLE VII.

THE ANALYSIS OF COVARIANCE ("r" VALUES) FOR DATA RELATING TO OAT ROOTS.

Factors	Sampling	r	n_2
Cu content, yield	3rd	−0·461 N.S.	14
Cu K	"	−0·721 **	14
" P	"	−0·596 *	14
" Ca	"	0·796 ***	14
K P	1st, 2nd, 3rd	0·685 ***	42
" Ca	3rd	−0·572 *	14
P Ca	"	0·393 N.S.	14

Symbol.	*P.*
N.S.	>0·05
~	0·05
*	0·02
**	0·01
***	0·001

Tables IV and VII indicate a significant correlation between K and P in oat tops and roots respectively. To determine whether the correlation was influenced by the amount of copper applied to the plants, "r" values were calculated for each rate of copper, using data for the three samplings. The results are shown in Table VIII.

TABLE VIII.

THE EFFECT OF CU TREATMENT ON THE CORRELATION COEFFICIENTS ("r" VALUES) FOR K AND P IN OAT.

Plant portion	Cu_0	Cu_1	Cu_2	Cu_3	n_2
Tops	0·941 ***	0·902 ***	0·922 ***	0·887 ***	14
Roots ..	0·277 N.S.	0·710 ***	0·523 ~	0·800 ***	14

Symbol.	*Probability.*
N.S.	>0·05
~	0·05
*	0·02
**	0·01
***	0·001

The correlation was significant at all rates of Cu in oat tops. It was also significant in the roots of plants given Cu, but not in untreated plants (Cu_0). Thus Cu appears to affect the K-P correlation in roots, but in tops the two nutrients are significantly correlated in the absence of Cu treatment.

Fig. 1 shows the effect of Cu treatment on the Cu contents of oat tops and roots respectively. With increasing addition of Cu to the soil, Cu accumulated proportionately more in the tops than in the roots.

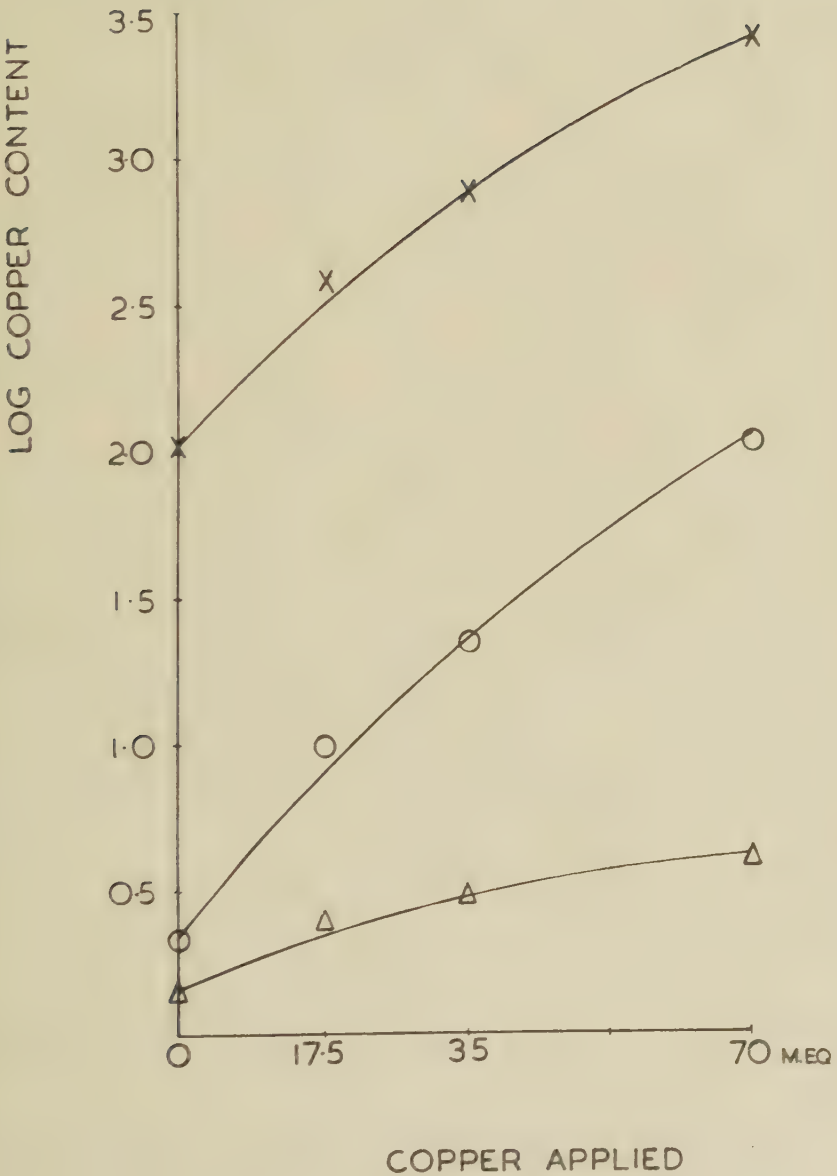

Fig. 1.

The effect of Cu on Cu status of oat tops and roots.

X ———— X Roots.

O ———— O Tops.

Δ ———— Δ Tops. Roots.

The effect of Cu application on the Ca, P and K contents of oat roots is shown in Fig. 2.

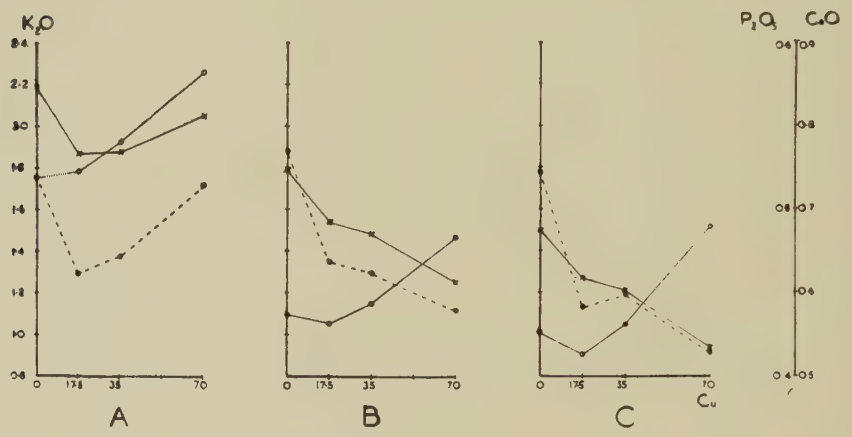

Fig. 2.

Effect of Cu on the mineral status of oat roots.
A, B and C represent 1st, 2nd and 3rd Samplings, respectively.

X ———— X K_2O.
● — — — ● P_2O_5.
O ..... O CaO.

1951 Experiments.

Experiment E, oat. The incidence of iron deficiency and mortality rates for oat recorded four days before sampling, are given in Table IX.

TABLE IX.

Mortality Rates and Iron Deficiency in Oat.

M.eq. Cu applied per pot	0	12·5	25	50
Mortality —% of original population ..	0	1	33	83
Iron deficiency— „ „ „ ..	0	6	19	7

Iron deficiency symptoms were observed mainly in the plants receiving 25 and 50 m.eq. Cu. (Plate V—Fig. 2). At the highest Cu level the mortality rate was 83%, hence the low % of plants showing iron chlorosis, as the latter value is based on the original number of plants per treatment. Oat grown in sterilised soil did not show symptoms of iron deficiency when treated with copper at the same rates as for the unsterilised soil.

Experiment F, rye and barley. No symptoms of iron deficiency were observed. The mortality rates and visual symptoms recorded three days before sampling are given in Table X. Percentages are expressed on the original number of plants per treatment.

TABLE X.

Mortality Rates and Visual Symptoms in Rye and Barley.

M.eq. Cu applied per pot	Rye (R) Barley (B)	0	5	10	20
Mortality	R	0	0	0	51
	B	0	0	11	83
Yellowing or necrosis ..	R	0	7	28	50
	B	0	2	44	14
Purple tinting	R	0	4	22	21
	B	0	0	8	0

A comparison of the mortality rates shows that Cu is more toxic to barley than to rye. Yellowing or necrosis developed in the older leaves of both crops but no symptoms were observed in the younger leaves of plants given Cu. A few barley plants showed purple tints at the base of the stem. Tinting occurred more frequently in rye, at all rates of Cu, and was confined to the older leaves.

Experiment G, sugar beet and kale. Copper was applied at 0, 10, 20 and 40 m.eq. per pot respectively. A few severely stunted plants at the highest rate of Cu developed an intervenal chlorosis in the older leaves, or in some instances in all leaves. Two days before sampling, the mortality rates for beet at 20 and 40 m.eq. Cu were 2 and 57%, and those for kale 1 and 58%. The effect of copper on the two crops is thus seen to be similar.

TABLE XI.

The Effect of Copper on Mortality Rates and Iron Deficiency in Oat and Barley.

Experiment	M.eq. Cu per pot	Mortality	Fe deficiency
Oat—August, 1950	70	10	Nil
Oat—June, 1951	12·5	1	6
	25	33	19
Barley—August, 1951 ..	10	11	Nil

It appears from Table XI that the onset of Fe deficiency symptoms is influenced by the time of year. Iron deficiency symptoms were observed in oat in June, but in August using greater amounts of Cu there was no visible effect on iron metabolism. Similar soils were used in all three experiments.

Summary.

(1) K, Ca, Mg, P, Fe, Mn and Cu status and yield data are given for tomato and oat grown in soils treated with copper at various milli-equivalent levels. In addition, some effects of Cu on the growth of oat, rye, barley, sugar beet and kale are described.

(2) In tomato a total of 563 m.eq. Cu significantly decreased the yield of fruit and increased the K, Mg and Cu contents of mid-stem leaves. Cu and Fe were not significantly correlated, but correlations were observed for (1) Cu and K, (2) Mn and P, in mid-stem leaves.

(3) In oat, 70 m.eq. Cu significantly decreased the yield of tops and (at the 2nd Sampling only) of roots. Cu application did not affect the Ca, K and P status of tops, but in roots Ca was higher, and K and P lower in plants given Cu. Positive correlations were observed for K and P in both tops and roots ($P = 0{\cdot}001$), and for (1) Cu and Fe ($P = 0{\cdot}05$), and (2) Fe and Mn ($P = 0{\cdot}001$) in tops, and for Cu and Ca in roots ($P = 0{\cdot}001$). Negative correlations were observed in roots for (1) Cu and K ($P = 0{\cdot}01$), and (2) Cu and P ($P = 0{\cdot}02$).

(4) The results of the 1951 experiments confirm those of 1949 and 1950 in showing that it is difficult to reproduce iron deficiency symptoms in crop plants treated with copper, in soil culture. Contrary to sand and water culture experiments, problems in relation to fixation, saturation and subsequent release of Cu in soil play an important part.

Acknowledgments.

Thanks are due to Dr. D. J. D. Nicholas for his interest and encouragement throughout these experiments. Thanks are also due to Mr. G. H. Jones, A.R.P.S., who made the photographic records, to Miss O. M. Woodhouse who assisted in recording visual symptoms, and to Mr. M. A. Wright for assistance with some of the copper analyses.

APPENDIX.

Washing experiment with oat.

In describing the effects produced in oat roots by the application of Cu, mention was made of the differences in P and K analyses on duplicate samples at the 3rd Sampling. The following experiment was made to ascertain whether this difference might be due to a slight variation in the washing procedure applied to the two sets of samples.

Forty-eight 6″ pots of compost were sown with oat (var. S 84); 10 seeds per pot. The plants were harvested when 8″ high, the pots being divided at random into two sets of 24. The roots in each set were washed by a different team of workers, then dried, weighed, and analysed for P. The washing technique used was similar to that employed in experiments using phosphorus-32 (2).

The two mean values, 1·37 and 1·47% P_2O_5, for the two sets of plants, differ significantly at $P = 0{\cdot}05$. The lower value is associated with the plants which received on average the more prolonged washing.

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THE CONTROL OF MOLYBDENUM DEFICIENCY IN LETTUCE UNDER FIELD CONDITIONS.

By WILLIAM PLANT.

Molybdenum deficiency in lettuce has been reported by Brenchley and Warington (1) in water culture experiments, and Wilson (2) has shown that a growth response was obtained in the field by applying a solution of sodium molybdate to the foliage of chlorotic lettuce.

During the summer of 1950, lettuces, growing on an acid soil of the Lower Greensand, were observed to be markedly stunted and chlorotic. Trials on correcting molybdenum deficiency in cauliflower (3) at the same centre had been successful and it was assumed that the symptoms shown in the lettuce were due to molybdenum deficiency. Nitrogen in available form had been added liberally and the possibility of a deficiency of this element had been thereby eliminated.

An experiment to test the assumption was carried out in 1951 and the results confirmed that the lettuce plants were suffering from molybdenum deficiency.

Experimental lay-out.

The experimental plots had been laid out initially for a cauliflower trial on new arable ground (pH 5·2) and the lettuce crop was the second to be grown on the experimental site.

Seven treatments, replicated threefold, were given as follows :—

(1) Control.
(2) Gypsum, 3 tons per acre (applied 20/2/50).
(3) Gypsum, 3 tons per acre (applied 20/2/50) ; followed by sodium molybdate at 2 lb. per acre (applied 27/2/50).
(4) Ground limestone, 3 tons per acre (applied 20/2/50).
(5) Ground limestone, 6 tons per acre (applied 20/2/50).
(6) Sodium molybdate, 2 lb. per acre (applied 27/2/50).
(7) Sodium molybdate, 4 lb. per acre (applied 27/2/50).

A basal dressing of ammonium phosphate and muriate of potash had been applied (20/2/50) over the whole experimental plot.

The molybdenum contents of the ground limestone and gypsum were found to be small and incapable of correcting the deficiency.

Lettuce (var. Borough Wonder) was sown 1/3/51 under Dutch lights, adjacent to the experimental plot. Planting out commenced 29/3/51, and the plants were cut for market from 20/6/51 to the end of that month. A wireworm attack of considerable severity developed in the early stages but diminished as the plants grew away in May.

Visual symptoms.

The syndrome of molybdenum deficiency (see Hewitt (4)) was not noticeable until early May when the general growth rate began to increase. By the end of May a large proportion of plants had been killed by the deficiency ; those that remained tended to show a recovery in June previous to the plants being cut. In the early stages of the deficiency the plants were chlorotic and

stunted. The lamina failed to develop and the leaves remained ovate rather than round as in a healthy plant. Chlorosis of the leaf was followed by necrosis of the margin. The texture of the leaf was " papery " and almost translucent ; the necrotic areas were light brown (Plate VI, Fig. 1). The plants failed to heart (Fig. 2) where the deficiency was severe and eventually shrivelled and died. The syndrome described occurred in treatments 1, Control ; and 2, Gypsum.

Yields and chemical data.

The yields and molybdenum and ascorbic acid contents are shown in the following table. Leaf samples were collected on 6/6/51 for the chemical analyses.

Yields and Molybdenum and Ascorbic Acid Contents of Lettuce.

Treatment	Soil pH	Yield cwt. per acre	Mo p.p.m. in dry matter	Ascorbic acid mg/100 gm. fresh wt.
1. Control	5·2	32	0·06	50
2. Gypsum, 3 tons p.a.	4·9	24	0·06	51
3. Gypsum, 3 tons p.a. + Sodium molybdate, 2 lb. p.a.	5·0	62	0·10	57
4. Ground limestone, 3 tons p.a. ..	6·3	59	0·09	65
5. Ground limestone, 6 tons p.a. ..	6·4	56	0·14	60
6. Sodium molybdate, 2 lb. p.a. ..	5·4	45	0·08	63
7. Sodium molybdate, 4 lb. p.a. ..	5·2	64	0·08	55
S.E.	—	—	0·038	9·32

The mean yields from treatments receiving either lime or sodium molybdate at different rates were more than doubled compared with those for Control and Gypsum. The lowest yield of 24 cwt. per acre given by gypsum was almost trebled by the addition of sodium molybdate at 4 lb. per acre.

The molybdenum contents of the plants from the different treatments did not differ so markedly as the yields. It is noticeable, however, that the lowest values, 0·06 p.p.m., were found in the control and gypsum, whilst the highest figure 0·14 p.p.m. occurred in the ground limestone treatment at 6 tons per acre. It appears from these results that symptoms of molybdenum deficiency can be associated with values of 0·06 p.p.m. molybdenum. It should be realised that although treatments incorporating either ground limestone or sodium molybdate only raised the molybdenum content on an average, to 0·10 p.p.m., the amounts removed from the soil by their greater crop weights would be appreciable.

The values for ascorbic acid show little change due to the treatments. This result is in contrast to the values of ascorbic acid in molybdenum deficient cauliflowers (3) which showed a significant decrease compared with healthy material.

Fig. 1. Molybdenum deficient lettuce.
Note the translucent texture of the ovate leaves and necrotic leaf edges.

(*a*) (*b*)

Fig. 2 (*a*) Molybdenum deficient lettuce.
Note lack of laminal development and hearting.
(*b*) Lettuce from treatment with sodium molybdate, 4 lb. per acre.

Summary.

(1) An experiment with lettuce was carried out on an acid Lower Greensand soil (pH 5·2) known to produce molybdenum deficiency in cauliflower.

(2) Treatments designed to vary the level of molybdenum were given consisting of ground limestone, sodium molybdate and gypsum.

(3) The syndrome of molybdenum deficiency observed in the lettuce crop comprised chlorosis, necrosis, stunting of growth, and lack of "hearting." These symptoms occurred mostly on the Control and gypsum treatments.

(4) The deficiency was corrected by the limestone treatments and by sodium molybdate at 2–4 lb. per acre but not by gypsum.

(5) Leaf analyses were carried out to determine the molybdenum and ascorbic acid values in the different treatments. Molybdenum deficient lettuce leaves contained 0·06 p.p.m. molybdenum and healthy leaves 0·08–0·14 p.p.m. molybdenum.

Acknowledgments.

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Thanks are due to Mr. J. Redmond for carrying out the molybdenum estimations and to Dr. S. C. Agarwala for determining the ascorbic acid values.

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THE SPREAD OF *ARMILLARIA MELLEA* IN APPLE ORCHARDS.*

By R. W. MARSH.

In 1938, the Horticultural Committee of the (then) Provincial Advisory Council of the Bristol Province recommended a survey of farm orchards in Somerset to explore the possible effects of variety, age, rootstock and soil on liability of apple trees to infection by the root rot fungus, *Armillaria mellea*. The orchards selected were chosen by Mr. J. E. Forshaw (now N.A.A.S. Horticultural Advisory Officer for Somerset), whose invaluable assistance throughout this investigation is gratefully acknowledged. When it became clear that the pattern of infection by *Armillaria mellea* could not be elucidated without long-term recording, four Somerset orchards were mapped in detail in 1938 by Mr. Forshaw, Dr. E. H. Wilkinson and the author. Annual inspection of these orchards was maintained until 1940, when recording lapsed on two of the sites. The other two were visited annually until 1951 and form the source of the observations reported in this paper. These will be referred to below as the Taunton and Glastonbury orchards respectively. Both are grass orchards of standard apples on seedling stocks.

Sites and Methods.

The Taunton orchard. This orchard, of approximately 100 × 80 yards, on a well-drained sandy loam, contained in 1938 104 living trees and 18 dead trees and stumps. The planting distances varied between 16 ft. 6 in. and 31 ft. (see Text–fig. 2). The orchard included not fewer than twenty varieties (cider, culinary and dessert). The sorts most commonly represented—Morgan Sweet (21 trees), Kingston Black (12 trees) and Blenheim Orange (11 trees)—were generally distributed through the orchard. The tree ages ranged from 2 to approximately 80 years : a broad classification into age groups is given in Table III.

The Glastonbury orchard. The Glastonbury orchard, 230 × 53 yards, lay on the margin of the Somerset "moors," about 40 ft. above sea-level, on heavy soil with a high water-table. In 1938 it contained 157 living trees and 7 dead trees and stumps. The planting distance varied widely but was usually of the order of 20 ft. (see Fig. 1). The identified trees belonged to 12 varieties, the commonest being Red Jersey (24 trees) and Bramley (36 trees), both of which were distributed throughout the orchard. Most of the Bramleys were newly-planted : otherwise the age distribution was similar to that in the Taunton orchard (see Table III).

Method of recording. Records were ordinarily taken in September-October, when premature defoliation sometimes provided a guide to the presence of *Armillaria* infections. Any tree showing unthrifty foliage, an unusually leaning stem or a suspicious appearance of the bark at the butt of the trunk was carefully examined. Soil was removed to a depth of about six inches to bare the upper central portion of the main roots, which were

* Slightly abridged from an article appearing in *Transactions of the British Mycological Society*, vol. 35, pt. 2.

then explored by incisions made into the cortex and wood. Usually it was not practicable to examine any part of the root system except that immediately adjoining the base of the trunk. Blackening of the host tissues was not recorded as an infection unless it was accompanied by the typical white fan-shaped mycelial plates of *A. mellea*. It frequently happened that there was a delay of two or three years between the first recording of infection and the death of the tree and, in this interim period, identification of the fungus could be confirmed by the presence of rhizomorphs and, occasionally, of sporophores.

Results.

Yearly record of infections.

Text-figures 1 and 2 show the positions of each tree newly recorded as infected with *Armillaria mellea* in each of the years 1938 to 1951, except 1950. The yearly record for the two orchards is summarised in Table I.

The infections credited to 1938 include a number of dead trees and stumps representing the accumulated effect of the attacks for several years immediately preceding the first (1938) recording. In the Taunton orchard, in particular, such attacks largely account for the patch of infection shown in Fig. 2 near the eastern side of the orchard. The 1939 figures may also include some infections that should have been recorded in 1938 but were overlooked. After 1939 it is thought probable that the figures fairly indicate the progress of infection in the preceding 12 months. On Text-figs. 1 and 2 the infections existing more than ten years before these diagrams were drawn (in 1951), are emphasized by being shown in white on a black background.

Table I gives no consistent evidence of seasonal effect, when the two orchards are considered together: neither does it indicate that the wetter Glastonbury site exhibits any outstandingly greater liability to *A. mellea* infection than the Taunton orchard.

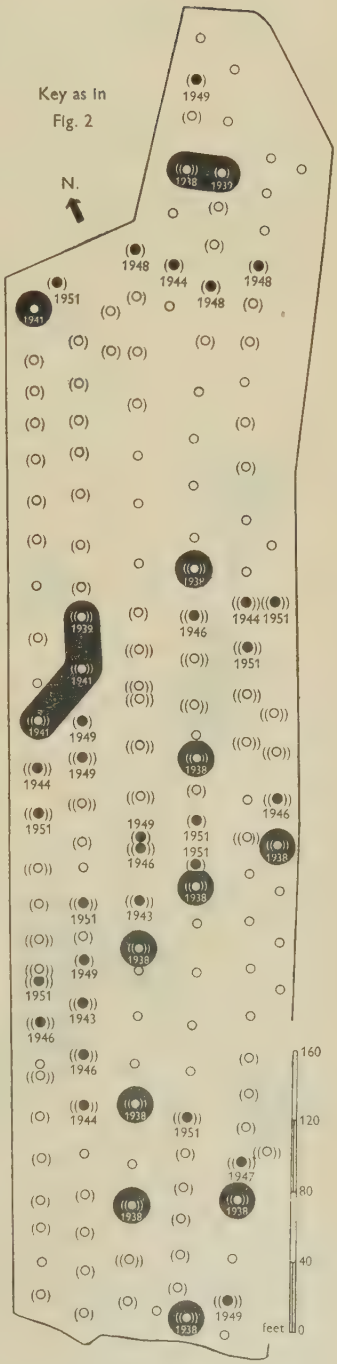

Fig. 1.
Glastonbury orchard

TABLE I.

NUMBERS OF TREES NEWLY INFECTED WITH *A. mellea*.

	1938	'39	'40	'41	'42	'43	'44	'45	'46	'47	'48	'49	'51	Total
Taunton ..	16	9	1	2	0	3	1	1	0	1	1	6	1	42
Glastonbury ..	10	2	0	3	0	2	4	0	5	1	3	6	9	45

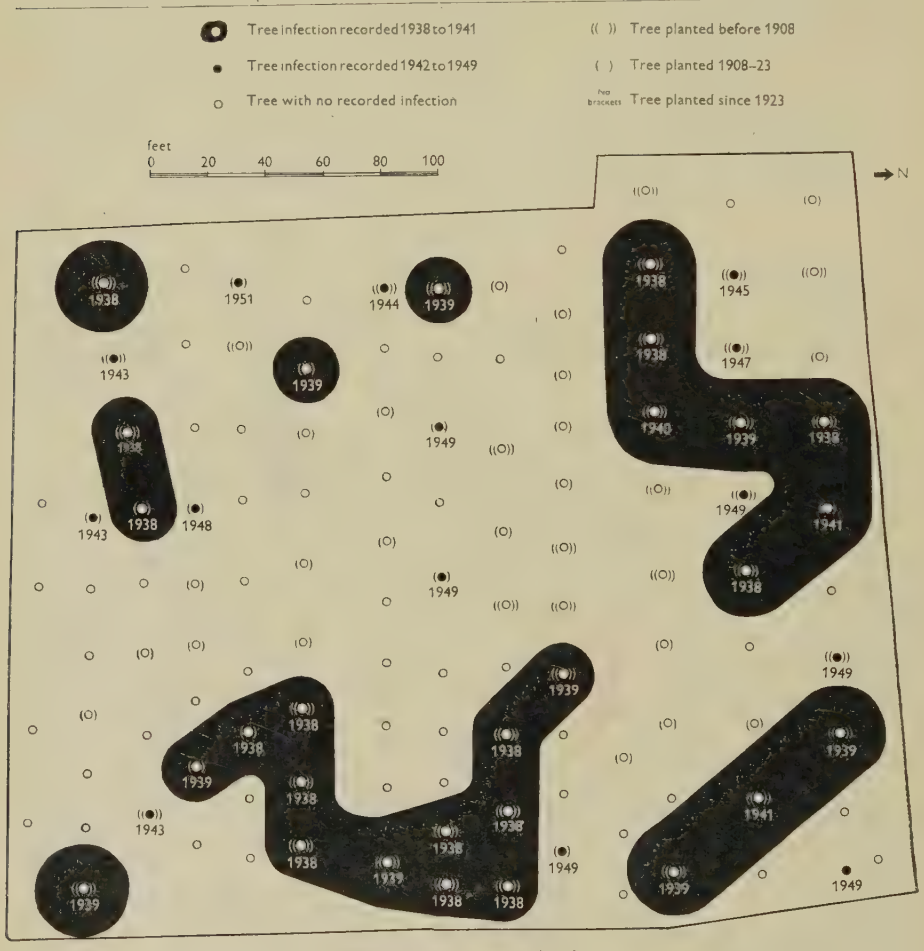
Tree infection recorded 1938 to 1941
Tree infection recorded 1942 to 1949
Tree with no recorded infection
(( )) Tree planted before 1908
( ) Tree planted 1908–23
No brackets Tree planted since 1923
feet
0 20 40 60 80 100
N

Fig. 2. Taunton orchard

Susceptibility of varieties.

A detailed statement of infections classified under varieties is not given, since the results so far obtained show no indication of significant differences in varietal susceptibility. For example, in the Taunton orchard, the trees of Morgan Sweet, making up 17% of the orchard population, have given 13% of the infections : in the Glastonbury orchard 15% of the trees are of the variety Red Jersey and this variety accounts for 17% of the infections. The apparent anomaly is provided by the Bramley trees at Glastonbury, which

form 22½% of the population but have contributed only 3% to the casualties. However, as already stated, these are almost all young trees, and this, as shown below, is a factor of over-riding importance.

Effect of proximity to existing infections.

The orchard plans (Text-figs. 1 and 2) show that new infections are more common in the vicinity of previous attacks, although the relationship between infection and proximity is obviously not clear-cut. Taking the 1939 orchard population and setting out, arbitrarily, zones of 35 ft. and 70 ft. from the infections then existing, the totals of trees in these zones that became infected in the ensuing twelve years are as shown in Table II.

TABLE II.

Effect of Proximity to Existing Infections.

Orchard	Distance from tree infected 1938-39	Total trees in 1939	Total infections 1940-51
Glastonbury ..	Under 35 ft.	49	11
	35–70 ft.	67	20
	Over 70 ft.	32	2
Taunton ..	Under 35 ft.	65	12
	35–70 ft.	29	5
	Over 70 ft.	nil	—

In the Taunton orchard all the trees were within 70 ft. of a 1938-39 infection. The figures from Glastonbury suggest a marked falling-off in infection beyond the 70 ft. range, but none of the records indicate that liability to attack in the 0–35 ft. circle was higher than in the 35–70 ft. zone.

Effect of tree age.

Exact figures for tree age could not be obtained, but in the 1938 survey, the three observers drew up an agreed list classifying each tree into one of three age categories : under 15 years, 15-30 years and over 30 years.

The incidence of *Armillaria* attack on trees of these three categories is summarized in Table III. Under the heading "Records 1938–39," the numbers of trees and of infected trees at the outset of the observations are assembled. The fate of the trees not recorded as infected in 1939 is followed in the remaining columns. (Because of the proximity effect shown in Table II, the trees more than 70 ft. from an original infection are shown separately).

Table III clearly indicates the effect of tree age on liability to infection. Of the tree population of 1938, those planted after 1923 have shown only 2% deaths compared with approximately 60% for the old trees. In the trees of medium age, the figure for the Glastonbury orchard is probably affected by the factor of distance from source of infection, but even so it is over 20%.

TABLE III.

Summary of Records of *A. mellea* Infections in Relation to Age of Tree.

Orchard	Tree planting date	Records 1938-39		Records 1940-51				Total all infections
				Trees < 70 ft. from 1938-39 infection		Trees > 70 ft. from 1938-39 infection		
		No. of Trees	Infections recorded	No. of trees	Infections recorded	No. of trees	Infections recorded	
Taunton ..	Before 1908	37	20	17	9	—	—	29
	1908–23	33	5	28	7	—	—	12
	1923–38	49	0	49	1	—	—	1
	Totals	119	25	94	17	—	—	42
Glastonbury	Before 1908	48	10	35	19	3	1	30
	1908–23	67	2	44	12	21	0	14
	1923–38	45	0	37	0	8	1	1
	Totals	160	12	116	31	32	2	45

If the records of 1938-39 are omitted, those of the succeeding 12 years still show over half of the old trees becoming infected compared with 2/94 of the young trees and 19/93 of the intermediate group.

Table III is incomplete in that it does not include trees planted since 1938 as replacements for those killed by *A. mellea*. In all, twenty-two such re-fills have been planted in the Glastonbury orchard and thirteen at Taunton. Although most of these young trees have been set out on the stations from which *Armillaria*-infested trees have been uprooted, not one has up to the present been recorded as attacked by *A. mellea*.

Discussion.

The observations in the Taunton and Glastonbury apple orchards were severely restricted by the inaccessibility of most of the tree roots. At these sites, a tree could not be listed as infected until the fungus mycelium had reached the trunk. Numerous examples were recorded in which infection was first noted in one segment of the base of the trunk, but complete encirclement required a further two or three years. This slow progress of the fungus in the tissues suggests that in these trees an invasion of the peripheral portions of the root system might precede by some years the recognition of visible symptoms of attack at the base of the trunk.

The apparent lack of any consistent pattern of spread in the infections in the Somerset orchards might therefore result from differences in the rate

of root invasion of individual trees adjoining an infected site. In conifers, variation in resistance to *A. mellea* has been recorded by Day (1929), but he suggested that external factors were of more significance than inherent qualities of the host. In the Taunton and Glastonbury orchards, however, the rarity of trunk infection in the young apples and its much more frequent occurrence in the older trees in the same environment suggest the hypothesis that the invasion of seedling apple roots is a gradual process in which the balance in favour of *A. mellea* increases as the vigour of the tree declines. In the younger tree, the normal development of new roots may compensate for the damage inflicted by *Armillaria* invasion ; as the tree matures it appears that the fungus gains the ascendancy ; and in trees over thirty years old there is a high probability that *Armillaria* infection of the roots will spread to the base of the trunk and bring about death of the tree by girdling.

It must be emphasized that the ages cited in the preceding paragraph are those of apple trees on seedling stocks, which have normally a profitable life of approximately seventy years (Barker, 1937). In commercial plantations of dessert or culinary apples on vegetatively-propagated semi-dwarfing stocks the life cycle is shorter and, on such stocks, trees not more than ten years old may be killed by *A. mellea*.

Because of the potential longevity of the standard apple tree, the losses due to *A. mellea* attack remain of serious economic importance, even if the tolerance to infection of the younger trees proves to be of general occurrence. In considering the possibility of assessing the results of control measures in such orchards, the observations given in this paper may serve as a warning that the incidence of individual new infections of *A. mellea* in relation to those already charted cannot be certainly predicted and, consequently, claims for the beneficial effects of trenches or similar barriers may be difficult to substantiate.

Acknowledgments.

The major contributions made by Mr. J. E. Forshaw and Dr. E. H. Wilkinson have already been noted. In addition, the help of Mr. S. H. Crowdy, Dr. R. S. Willison, Dr. R. J. W. Byrde and Mr. A. H. Fielding in the post-war recording is gratefully acknowledged. The blocks for the text-figures were kindly lent by the Cambridge University Press, and these figures are reproduced by permission of the editors of the *Transactions of the British Mycological Society*.

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SPRAYING EXPERIMENTS AGAINST APPLE AND PEAR SCAB AT LONG ASHTON, 1951.

By R. J. W. BYRDE and R. W. MARSH.

The experiments described in this paper form a continuation of those carried out at Long Ashton in 1949 and 1950 (Byrde and Marsh, 1951). The fungicidal and phytotoxic effects of materials showing promise in these earlier trials were further tested, together with those of thiocarbamates and organo-mercury sprays. The standard lime-sulphur programme was again used for comparison.

Plots.

The apple trials were made on Plot 23 in the Long Ashton Research Station plantations on the varieties Cox's Orange Pippin, Worcester Pearmain, Lane's Prince Albert and Stirling Castle. The trees, planted in 1935, were in grass, regularly cut by a gang-mower, and were spaced at 21 ft. except the Worcesters which were at 15 ft.

The pears used were two 25-tree rows, of William's Bon Chrétien and Doyenné du Comice respectively, planted at 15 ft. in grass. The original trees were set out in 1925 but there were many younger replacements.

Spray Materials.

The spray materials used may be briefly specified as follows :—

Lime sulphur. A commercial concentrate, used at 2% pre-blossom and 1% post-blossom.

Thiram. An 80% preparation of tetramethylthiuram disulphide made up for spraying at 3 lb. per 100 gal.

Ziram. An 80% preparation of zinc dimethyldithiocarbamate, used at 3 lb. per 100 gal.

Phenyl mercury chloride. A 2½% preparation used at 1¼ lb. per 100 gal. to give a spray containing 0·002% Hg.

Phenyl mercury acetate. A preparation of 2½% Hg content used at 12·8 oz. per 100 gal. to give a spray containing 0·002% Hg.

Diphenyl mercuric salt of di–2:2′–naphthylmethane–3:3′–disulphonic acid. A preparation containing 0·8% Hg diluted to 1 qt./100 gal to give a spray containing 0·002% Hg.

Bis(2–hydroxy–5–chlorophenyl) sulphide. (CR.305). A 20% preparation diluted to 0·1% for spraying.

Heptadecyl glyoxalidine. (341.SC). The concentrate, containing 34% heptadecyl glyoxalidine, was used at 3 pt. per 100 gal., with 8 oz. hydrated lime added.

Trichloromethylthiotetrahydrophthalimide. (SR.406). This phthalimide spray was employed as a 50% preparation, used at 2 lb. per 100 gal.

The sprays were applied by a small mobile outfit with a single-nozzle broom (disc 5/64 in. aperture ; pressure 75 lb./sq. in.).

Lay-out of experiments and spraying dates.

Worcesters (Exp. A5). The following eight one-tree treatments were randomised in each of four blocks on Plot 23 : thiram 0·3%, ziram 0·3%, chlorophenylsulphide 0·1%, phenyl mercury acetate (at 0·002% Hg), phenyl mercury chloride (at 0·002% Hg), soluble organo-mercury sulphonate (at 0·002% Hg), lime sulphur (at 2% pre-blossom and 1% post-blossom) and control. Three applications were given ; pre-blossom on 11th May and post-blossom on 4th June and 4th July.

Worcesters (Exp. A2). The following three treatments were randomised in each of nine 3-tree rows on Plot 23 : phthalimide spray (50%) at 2 lb. per 100 gal. ; lime sulphur at 2% pre-blossom and 1% post-blossom ; control. One pre-blossom spray was given on 7th May and two post-blossoms, on 5th June and 3rd July.

Cox (Exp. A1). A block of thirty trees was divided into five equal strips treated respectively from east to west, with heptadecyl glyoxalidine, no spray, lime sulphur, no spray and heptadecyl glyoxalidine. Lime sulphur was used at 2% pre-blossom and 1% post-blossom ; glyoxalidine at 3 pt./100 gal. The pre-blossom spray was on 3rd May and the post-blossom sprays on 5th June and 14–16th July.

Lane's P.A. and Stirling Castle (Exp. A3). This was a phytotoxicity trial on nine trees of each variety allocated as follows :

Controls, 3 trees ; heptadecyl glyoxalidine, 3 trees ; phthalimide, 3 trees. The strengths used were as in Exp. A1 and A2 and the applications were made on 3rd May, 6th June and 3rd July.

Doyenné du Comice and William's B.C. Each variety was divided into four 6-tree blocks within which the following three treatments were randomised on single trees : heptadecyl glyoxalidine, the phthalimide preparation and no spray. Sprays were applied on 24th April, 21st May and 11th–12th June.

Notes on weather and method of recording.

The summer of 1951 was characterised by a wet May and a very wet August. The monthly rainfall totals recorded at Long Ashton were (in inches) : April 3·80, May 2·94, June 0·68, July 1·66, August 5·10, September 3·02, October 0·75.

Mean percentage scab areas per leaf and per fruit were assessed by Tehon and Stout's method as amplified by Marsh (1947). The samples taken comprised at least 250 leaves, or 100 fruits, per tree, picked from a cone-shaped space extending to the centre of the tree from the north side. For the assessment of scab on pear fruits, where infection occurred as numerous small spots scattered over the fruit surface, models were prepared showing 0·5, 1, 2, 5, 10 and 20% infection of the total area.

Results.

Apples : Scab control.

Worcesters (Exp. A5). Mean percentage areas scabbed were assessed on leaves on 9th–13th August and on fruit on 23rd–25th August. Results are assembled in Table I.

TABLE I.

Results on Worcesters.

(Sprays applied 11th May, 4th June and 4th July)

Spray material	Concentration	Mean % area scabbed	
		Leaves	Fruits
Ziram (80%)	0·3%	0·04	0·02
Lime sulphur	2% then 1%	0·20	0·03
Thiram (80%)	0·3%	0·13	0·07
Soluble organo-mercury sulphonate..	0·002% Hg	0·64	0·26
Phenyl mercury chloride	0·002% Hg	0·59	0·26
Phenyl mercury acetate	0·002% Hg	0·58	0·30
Chlorophenyl sulphide	0·1%	1·33	0·45
Control	—	1·64	0·78
Significant difference at $P = 0.05$		0·44	0·12

Worcesters (Exp. A2). The leaf assessments were made on 14th–15th August and the fruit assessments on 27th–28th August (see Table II).

TABLE II

Results on Worcesters

(Sprays applied 7th May, 5th June and 3rd July)

Spray material	Concentration	Mean % area scabbed	
		Leaves	Fruits*
Phthalimide spray (50%)	0·2%	0·11	0·07
Lime sulphur	2% then 1%	0·38	0·34
Control	—	0·95	0·80
Significant difference at $P = 0.05$		0·17	0·17

* On a sample of 50 fruits per tree.

Cox (Exp. A1). Scab was assessed, on fruits only, on 27th September–3rd October. Results are given in Table III.

The mean values for lime sulphur and heptadecyl glyoxalidine are both significantly lower than that for control but do not differ significantly from each other.

TABLE III

Results on Cox

(Sprays applied 3rd May, 5th June and 14th–16th July)

Spray material	Concentration	Mean % area scabbed : Fruits
Heptadecyl glyoxalidine (34%)..	0·375%	0·45
Lime sulphur	2% then 1%	0·49
Control	—	1·01

Apples : phytotoxicity.

Lanes. Stirlings. Cox. As in previous years (Byrde and Marsh, 1951), phytotoxicity was assessed numerically by fruit counts on the sprayed trees on 4th September (Stirlings). 26th September (Lanes), and 27th September–3rd October (Cox). The results of the 1951 trials are given in Table IV.

TABLE IV

Mean Numbers of Fruit per Tree Following Fungicide Treatments

Treatment	Variety		
	Lane	Stirling	Cox
Phthalimide	448	364	Not tested
Heptadecyl glyoxalidine ..	441	222	710
Lime sulphur	Not tested	Not tested	763
Control	455	341	569

Worcesters. Records were made of the numbers of russeted unscabbed fruits noted in picking the crop in Experiments A2 and A5. Results are given in Table V.

The high russeting figure following treatment with ziram is noteworthy.

Pears : scab control.

Williams. The crop harvested was extremely light, so the fruits for each treatment were bulked and the scab assessed on the totals on 20th August. (See Table VI).

The crop on the Comice trees was negligible, and no assessment of scab was possible.

TABLE V

RUSSETING OF UNSCABBED FRUITS OF WORCESTER PEARMAIN FOLLOWING FUNGICIDE TREATMENTS

Exp.	Treatment	Unscabbed fruits	
		Total	No. russeted
A2	Phthalimide	398	14
	Lime sulphur	244	17
	Control	76	7
A5	Chlorophenyl sulphide	77	8
	Soluble mercury sulphonate ..	107	11
	Phenyl mercury chloride ..	134	7
	Phenyl mercury acetate ..	125	4
	Thiram	182	29
	Ziram	193	41
	Lime sulphur	195	12
	Control	55	0

TABLE VI

RESULTS ON WILLIAMS

(Sprays applied 24th April, 21st May, 11th–12th June).

Spray material	Concentration	Percentage fruit area scabbed
Phthalimide spray (50%) ..	0·2%	0·36
Heptadecyl glyoxalidine (34%) ..	0·375%	1·33
Control	—	4·53

Discussion.

Rainfall at Long Ashton in 1951 was above the 1914–48 mean in May and August, equal to the mean in September and below in June and July. The season, therefore, although less favourable to apple scab than 1950, provided lengthy infection periods in May and August which satisfactorily tested the field performance of the fungicides under trial.

The mixed glyoxalidines formulation (341.C), which had been included in Long Ashton trials for the previous four years was omitted in 1951. It was however used (Croxall and Jenkins, 1952) in a Warwickshire field trial which demonstrated the ability of this fungicide to control pear scab satisfactorily in a commercial plantation of Doyenné du Comice. The newer heptadecyl

glyoxalidine preparation (341.SC) showed at Long Ashton in 1951 a fungicidal performance on Cox's Orange Pippin equal to that of lime sulphur without any apparent phytotoxicity.

The phthalimide spray (SR.406) maintained in 1951 the promise shown in the 1950 trials. Its fungicidal performance on Worcester Pearmain apples was significantly better than that of lime-sulphur, and in controlling scab on Williams' pears it was markedly better than heptadecyl glyoxalidine. No phytotoxic effect of this spray has been recorded in the trials at Long Ashton.

In another trial on Worcester Pearmain apples the two thiocarbamates, ziram and thiram, both ranked equal to lime sulphur in apple scab control, although showing higher figures for fruit russeting. In the same trial, three organo-mercurials, used at 0·002% Hg equalled one another in scab control but were less effective than lime sulphur. The mercury concentration used was below that generally recommended for organo-mercury sprays. The experimental material, chlorophenyl sulphide (CR.305), gave a poor field performance in this trial.

The results assembled in Table I may be compared with those found in a 1951 field trial in Worcestershire, described elsewhere (Croxall and Jenkins, 1952). In the Worcestershire trial the mean percentage fruit areas scabbed on Worcester Pearmain following the respective spraying treatments were : ziram 0·17, lime sulphur 0·25, mixed glyoxalidines 0·26, thiram 0·36, organo-mercury sulphonate 0·39, heptadecyl glyoxalidine 0·46, control 2·24. In this trial, also, the mercurial spray, and the thiram, were used at strengths below those generally recommended.

The trials in the West Midlands and those at Long Ashton illustrate the extending field of possible substitutes for lime sulphur in the control of apple and pear scab. Within this field, the grower's choice remains at present closely restricted by the limited availability of the newer fungicides.

Acknowledgment.

The help of Mr. A. H. Fielding in carrying out spray applications and in recording is gratefully acknowledged.

Summary.

On Worcester Pearmain apples at Long Ashton in 1951, the level of scab control obtained by three applications of lime sulphur was equalled by that following three applications of either 0·24% zinc dimethyldithiocarbamate (ziram) or 0·24% tetramethylthiuram disulphide (thiram). Less effective control was provided by three proprietary organo-mercury sprays used at 0·002% Hg. Russeting was noted on 16% of the fruits sprayed with thiram and on 21% of those sprayed with ziram.

In another trial on Worcester Pearmain, 0·1% trichloromethylthiotetrahydrophthalimide (SR.406) surpassed lime sulphur in scab control. This phthalimide spray was non-phytotoxic to the varieties Worcester Pearmain, Lane's Prince Albert and Stirling Castle.

A 34% preparation of heptadecyl glyoxalidine (341.SC), used at 0·375%, equalled lime sulphur in apple scab control but was less effective than the phthalimide spray against pear scab. This glyoxalidine preparation showed no phytotoxic effect on Cox's Orange Pippin and Lane's Prince Albert.

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THE EFFECT OF AGE OF WOUND AND OF WEATHER ON THE SUSCEPTIBILITY OF APPLE INJURIES TO INFECTION BY THE BROWN ROT FUNGUS.

By R. J. W. BYRDE.

The general etiology of the apple brown rot fungus (*Sclerotinia fructigena* Aderh. and Ruhl.) has been comprehensively reviewed by Wormald (1935), who stressed the significance of fruit injury as a precursor of spore infection. Recent studies by Moore (1950) have determined the relative importance of various wound-causing agents. Less is known, however, of the factors which control the infection of an injury once it has been made. This note describes preliminary experiments on :

(1) The effect of the age of a wound on its susceptibility to infection.

(2) The effect of climatic conditions on the rate of infection of fruit injuries in the field.

Effect of Age of Fruit Wounds on Susceptibility to Brown Rot Infection.

Sound freshly-picked apples of the variety Laxton's Superb were artificially wounded at fixed time intervals during the late summer. The instrument used resembled a small cork borer, the depth of its cut being limited to 1 mm. by a flange : the disc cut out, which was 2·5 mm. in diameter, was then removed with a needle. Both the needle and the disc-cutting instrument were sterilised frequently. The skin of each apple was punctured in ten or twenty places, and the fruit transferred to a moist chamber at laboratory temperature to await inoculation with a spore suspension of *S. fructigena*. The proportion of wounds to become infected was recorded forty-eight hours after inoculation. Table I summarises the results of three such tests : the inoculations were carried out on July 28th, August 11th and August 25th, 1950.

These figures suggest that under the conditions of this experiment an appreciable resistance to infection had developed in twenty-four hours from the time of wounding.

TABLE I.

THE EFFECT OF AGE OF APPLE WOUNDS ON THEIR SUSCEPTIBILITY TO BROWN ROT INFECTION.

Time between wounding and inoculation (hrs.)	Proportion of total wounds infected
0	49/50
1·5	30/30
6	30/30
24	17/50
96	2/50

Effect of Climatic Factors on Field Infection.

The trees used in this investigation comprised one long row and one short row of bush Laxton's Superb, separated by a row of Lane's Prince Albert. Tree spacing was 18 ft. × 18 ft. The experiment was initially designed as a spray trial, using applications of 0·05% dichloronaphthaquinone on half the trees in mid-July and mid-August, 1951. In early September it became clear that natural infection was too light to justify disease assessment, and the trees were therefore used for a preliminary study of the development of infection under different climatic conditions.

TABLE II.

BROWN ROT INFECTION OF INJURIES ON LAXTON'S SUPERB APPLES.

Date injured	Date examined	No. infections apparent from 80 injuries
Sept. 5	Sept. 10	67
,, 8	,, 12	33
,, 10	,, 14	50
,, 12	,, 17	29
,, 14	,, 19	14
,, 17	,, 21	8
,, 19	,, 24	0
,, 21	,, 26	16
,, 24	,, 29	3
,, 26	Oct. 1	0

On ten dates during September, five injuries, to simulate bird pecks, were made with forceps (sterilised each day before use) on one fruit on each of sixteen Laxton's Superb trees. No inoculum was added, and any brown rot which developed therefore did so from spores naturally present in the orchard.

Each injury was examined four or five days later, and infections recorded. Table II summarises the results obtained. There was no indication that the fungicide applications had reduced infection on sprayed trees.

Chi-square analysis of this data suggests that there are significant variations in infection between different days. Broadly speaking, infection was high at the beginning of the month, and low at the end. Figure 1 shows the infection data of Table II plotted against mean temperature over the incubation period, and suggests that infection rate may be correlated with temperature. Comparisons with rainfall and wind data showed a much lower degree of correlation.

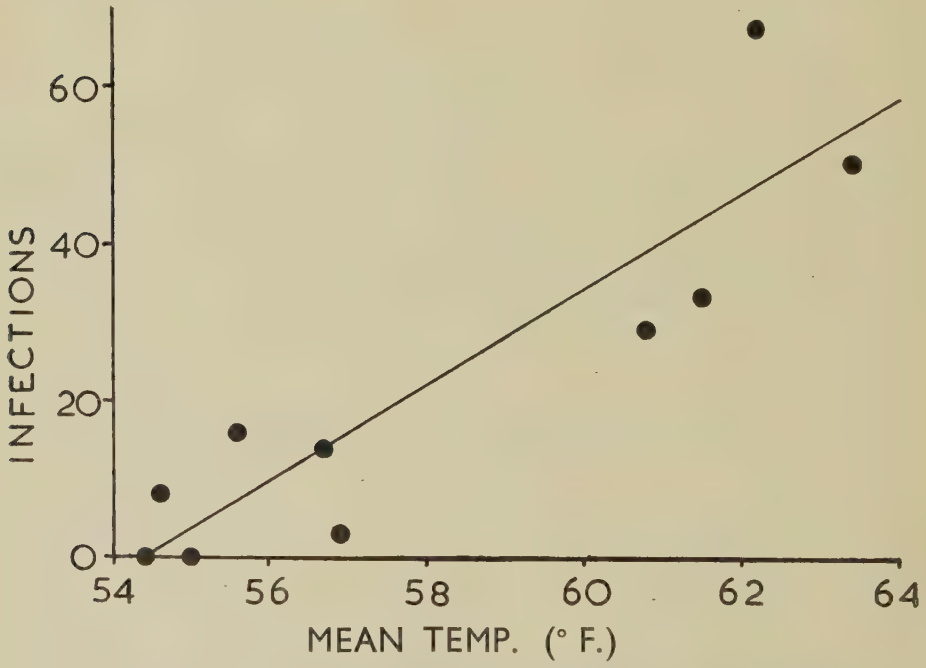

Fig. 1. Number of brown rot infections from eighty wounds, plotted against mean temperature over incubation period.

Discussion.

In the first experiment, the onset of host resistance may have been influenced by the temperature and humidity of the laboratory moist chambers.

The effect of weather in the second experiment may have been on spore production, spore dissemination, spore germination, mycelial spread or host reaction. Spore production is thought to be favoured by conditions of high humidity coupled with high temperature (Byrde, 1951), for example, while mycelial growth of the brown rot fungi is greatest at a temperature of about 25°C. (77°F.) (Wormald, 1935).

It is considered that the results reported in this paper are of a preliminary nature, and do not allow broad conclusions to be drawn. Further experiments are planned, and a more detailed consideration will be deferred until their results are known.

Summary.

Infection of apple fruits by the brown rot fungus, *Sclerotinia fructigena*, took place less readily on day-old injuries than on those freshly made.

Susceptibility of Laxton's Superb apples to brown rot infection in early September (mean temperature approx. 61.6°F.) was much higher than in late September (mean temperature approx. 55.7°F.).

Acknowledgments.

Meteorological data were kindly supplied by Mr. G. E. Clothier.

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TESTS OF ERADICANT FUNGICIDES AGAINST BROWN ROT. I.

By R. J. W. BYRDE and A. H. FIELDING.

This progress report deals with the evaluation of two organo-mercurial compounds and two chlorinated phenol derivatives as eradicant fungicides against *Sclerotinia fructigena* Aderh. and Ruhl., the principal fungus causing brown rot of apples and plums.

Experimental.

The fungicides tested were :—

(a) *Phenyl mercury chloride.* A stock suspension was prepared by grinding together :—

Phenyl mercury chloride (64% Hg)	10 gm.
10% sulphite lye	100 ml.

This was diluted to give the strengths listed in Table I. Estol "H" was added to the diluted solutions at 0·25%.

(b) *Diphenyl mercuric salt of di-2:2′-naphthyl methane-3:3′-disulphonic acid*, supplied as a water-miscible liquid containing 0·8% Hg, and incorporating a wetting agent.

(c) *Sodium pentachlorophenate*, as an aqueous solution, with tar-oil emulsion added to a final concentration of 4% oil.

(d) *Pentachlorophenol.* A stock emulsion was prepared from :—

Pentachlorophenol	20 gm.
Tar-oil	100 ml.
10% sulphite lye	100 ml.

The pentachlorophenol was dissolved in the tar-oil by warming, and the oil solution then emulsified. The concentration of tar-oil in the diluted pentachlorophenol emulsions was adjusted to 4% throughout by addition of the appropriate quantity of a 50% tar-oil stock emulsion.

On April 12th—13th, 1951, batches of twenty detached mummified apples were treated with a range of concentrations of each of these four compounds, by dipping each fruit in a beaker of fungicide for five seconds. The fruits were allowed to dry on cellulosed wire-netting trays, which were transferred to a rack structure in the open after a period of twenty-four hours indoors. One hundred untreated fruits served as Controls.

The number of sporing pustules of unit size (1 mm. in diameter) was estimated for each fruit on July 23rd, 1951.

Results.

(i) *Organo-mercurials.*

The organo-mercury derivatives were tested at four levels of Hg. Table I summarises the results obtained ; the comparable figure for the untreated fruits was 64·8.

TABLE I.

Pustule Production on Detached Mummified Apples Treated with Two Organo-Mercurial Fungicides.

Concn. (% Hg)	Mean no. pustules per fruit	
	Phenyl mercury chloride	Diphenyl mercury salt
0·2	1·7	0·7
0·1	5·5	3·5
0·05	15·6	18·5
0·025	30·0	39·9

Analysis of variance shows that, while concentration differences are significant, there is no significant difference between the two mercury preparations. The inhibition of sporulation at a concentration of 0·2% Hg was greater than 95% in each case.

(ii) *Pentachlorophenol and its sodium salt.*

This pair of compounds was tested at five dosages, ranging from 0·8% to 0·05%, expressed as equivalent concentrations of pentachlorophenol. Table II summarises the results obtained. The comparable figure for fruits treated with tar-oil alone was 25·5 ; that for untreated fruits was 64·8.

Analysis of variance shows that while concentration is a significant factor, there is no significant difference between the two compounds. Inhibition of pustule production of the order of 98% of that of untreated fruits followed the use of both compounds at concentrations equivalent to 0·8% and 0·4% pentachlorophenol.

TABLE II.

Pustule Production on Detached Mummified Apples Treated with Pentachlorophenol Derivatives in the Presence of 4% Tar-Oil.

Concn. (%)*	Mean no. pustules per fruit	
	Pentachlorophenol	Sodium pentachlorophenate
0·8	1·5	0·0
0·4	1·1	0·5
0·2	5·6	6·5
0·1	8·7	19·7
0·05	18·0	16·8

* as equivalent concn. of pentachlorophenol.

Discussion.

In previous tests, phenyl mercury chloride has proved to be a reliable eradicant fungicide for the inhibition of sporulation on mummified apple and plum fruits infected with *Sclerotinia* spp. (Marsh, 1947, Byrde, 1952). Unfortunately, however, effective concentrations are uneconomic. The diphenyl mercuric salt of di-2:2′-naphthyl methane-3:3-disulphonic acid is likely to share this disadvantage, since its eradicant efficiency appears equal to that of phenyl mercury chloride.

No difference in eradicant efficiency was demonstrated between the two pentachlorophenol compounds. Under certain conditions, however, such as when heavy rain follows soon after fungicidal treatment, it is possible that the phenol might be more satisfactory than its highly water-soluble sodium salt (Byrde, 1952). The cost of effective concentrations of both these materials, unlike the mercurials, is not prohibitive.

Summary.

1. In tests on the inhibition of sporulation of detached mummified apples infected with *Sclerotinia fructigena*, the diphenyl mercuric salt of dinaphthyl methane disulphonic acid showed the same order of eradicant efficiency as phenyl mercury chloride.

2. Similarly, no significant difference in eradicant action was demonstrated between pentachlorophenol and its sodium salt.

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THE ROLE OF ERADICANT SPRAYS IN THE CONTROL OF APPLE CANKER.

By R. J. W. BYRDE and S. H. CROWDY (Long Ashton Research Station), and F. A. ROACH (National Agricultural Advisory Service).

Apple canker, caused by the fungus *Nectria galligena* Bres., is a serious problem in many orchards and nurseries in the West of England. At present, the only control recommended for the disease consists of rigorous excision of infected tissues : routine fungicidal sprays appear to have little effect (Wormald, 1946). Excision of cankers serves a dual purpose : firstly, it prevents further damage resulting from the spread of the canker concerned, and, secondly, it removes a source of spores which might otherwise initiate fresh infections.

Unfortunately, however, such an operation is tedious and difficult. This paper* describes early experiments in the use of fungicidal sprays which fulfil the second function of excision described above, namely the suppression of spore inoculum on established cankers. Such " eradicant " fungicides have been used with success against certain fruit diseases in the U.S.A.

Studies on the development of apple canker (Wiltshire, 1921 ; Marsh, 1939 ; Crowdy, 1952) have indicated that there are two main paths of entry into the host, leaf-scars and pruning wounds. There appear to be two critical periods for the initiation of leaf-scar infections : at the time of leaf-fall and at bud burst. Pruning cuts show a natural resistance to infection after two months. Therefore, if control is to be effected by the use of chemicals suppressing sporulation, protection must be given at the critical periods in Autumn and Spring. Further, the spray must be non-damaging to trees with leaves still attached.

The few eradicant fungicides that have been successfully employed against other pathogenic fungi include monocalcium and sodium arsenites, sodium dinitro-*ortho*-cresolate and sodium pentachlorophenate. Byrde (1950) found each of these materials, and also phenyl mercury chloride, approximately equal in inhibiting the sporulation of *Sclerotinia fructigena* Aderh. & Ruhl. on detached, mummified plums. The effective concentration of phenyl mercury chloride was approximately sixty times that used in conventional foliage protectant sprays.

Materials and Methods.

The eradicant fungicides used in the following trials were phenyl mercury chloride and sodium pentachlorophenate. Copper fungicides have been shown to be highly toxic to *N. galligena* (Marsh, 1939) and some attempts were made to distinguish between eradicant and protectant effects by including conventional copper sprays. The spray materials were formulated as follows : *Phenyl mercury chloride*, by dilution to 100 gal. of a stock suspension formed by milling 3 lb. of the active chemical with 3 gal. of 10% sulphite lye. Oil emulsion or wetting agent was added to the dilute wash if required. In one experiment a proprietary wetting agent at a final concentration of 1% was

* A full account of the investigations summarised in this paper will be published elsewhere Byrde, Crowdy and Roach, 1953).

used instead of sulphite lye. *Sodium pentachlorophenate*, as a 1% or 0·5% aqueous solution. *Bordeaux mixture*, prepared in the usual manner.

All trials were carried out on the variety Worcester Pearmain.

The effects of treatments on the incidence of leaf-scar cankers were assessed by counting the cankers occurring on approximately 100 shoots on the treated trees. Usually the shoots selected were formed during the season immediately prior to spraying and the probable time at which infection took place could be related fairly accurately to the time of treatment. The results of these counts are presented as the mean numbers of cankers per 100 shoots.

Experimental Results.

Prevention of sporulation.

In trials of 0·3% phenyl mercury chloride preparations at Long Ashton and in a Gloucestershire orchard, it was found that the sporulation of cankers on sprayed trees was markedly less than that on control trees. Even fifteen months after the initial spray application, the inhibition of sporulation was still greater than 50 per cent. Sodium pentachlorophenate at 0·5% was less effective : two months after application, the inhibition of sporulation was approximately 70%.

The effect of suppressing sporulation on the incidence of leaf-scar cankers.

In 1949-50 the effects of conventional copper sprays and of excision of cankers were examined in the Gloucestershire orchard. Data on leaf-scar infection recorded the following summer are presented in Table I and confirm the value of excision and the relative ineffectiveness of Bordeaux mixture applied in the period November-April inclusive.

TABLE I.

The Effects of Copper Sprays and Excision on the Incidence of Leaf-Scar Canker.

Treatment	Mean leaf-scar cankers per 100 shoots	
	Cankers cut	Cankers not cut
Control A, unsprayed	13	60
Control B, unsprayed	24	47
Bordeaux mixture (16–24–100) November	10	71
,, ,, ,, ; January	23	50
,, ,, ,, ; March	15	72
,, ,, (6–9–100) April	23	61
,, ,, November and April	15	38

In 1950-51 0·3% phenyl mercury chloride, with 1% JD-2 oil emulsion, was tested as a potential eradicant fungicide. The effect of the spray on the

sporulation of the pathogen has already been mentioned. The subsequent effect on the formation of leaf-scar cankers is summarised in Table II. A comparable figure for trees pruned and cleaned in 1949-50 is included.

TABLE II.

The Effect of 0·3% Phenyl Mercury Chloride Sprays on the Formation of Leaf-Scar Cankers.

Treatment	No. leaf-scar cankers per 100 shoots (Aug. 1951)
Phenyl mercury chloride; 25/10/50	2·0
,, ,, ,, ; 28/3/51	3·3
,, ,, ,, ; 25/10/50 and 28/3/51 ..	3·4
Pruned and cleaned, 1949-50	3·6
Control A, unsprayed	5·7
Control B, unsprayed	5·9

Phytotoxicity.

No damage to mature leaves and shoots followed applications of 0·3% phenyl mercury chloride prior to leaf-fall. Young shoots and leaves of the second growth were sometimes killed. No damage followed applications of eradicant fungicides during dormancy.

Discussion.

The data presented show that it is possible in the field to reduce infection materially by reducing the amount of spore inoculum either by cutting out diseased tissue or by the application of eradicant sprays. Two materials, sodium pentachlorophenate and phenyl mercury chloride, have been used: both reduce the sporulation of cankers for some time after inoculation, phenyl mercury chloride being the more effective. The data show further that phenyl mercury chloride owes its effectiveness in reducing leaf-scar canker to the suppression of sporulation rather than to any protectant action since the results of spraying with this compound are comparable to the results obtained by cutting out diseased material and are far superior to the effects recorded with protectant copper sprays.

The most suitable time for the application of an eradicant fungicide is probably just before leaf-fall. The effect of the spray on sporulation appears to persist for several months, and a spray applied at such a time would thus cover the critical infection periods.

The economic value of including an eradicant spray against canker in normal orchard routine depends largely on the age of the trees. A routine pre-leaf fall spray would probably do much to protect the trees in nurseries and young plantings from a serious outbreak of canker and so would allow the main framework of the tree to be built up without hindrance. Eradicant spraying alone, however, is unlikely to be satisfactory in rejuvenating an old and

cankered orchard. Such treatment might prevent new infections but the existing cankers would retain living mycelium which might eventually ring and kill the infected branches. Therefore, spraying should be supplemented by excision of cankers.

These trials indicate the potential value of an eradicant spray in the control of apple canker but as the experiments have dealt almost entirely with one material tested on a single variety of apple, wider tests of phytotoxicity are required before the treatment can be generally recommended.

Summary.

Sodium pentachlorophenate and phenyl mercury chloride have been tested as eradicant sprays against apple canker. Both chemicals checked sporulation of *Nectria galligena* on cankers but phenyl mercury chloride was the more effective and reduced sporing over fifteen months after application.

The incidence of leaf-scar infection was diminished by treatments reducing sporulation, such as spraying with phenyl mercury chloride or the removal of diseased material from the tree. Bordeaux mixture used as a protectant winter spray was ineffective in reducing leaf-scar infection.

Acknowledgments.

The authors acknowledge with gratitude the assistance and co-operation of Mr. N. Rimmington, the owner of the trial orchard in Gloucestershire, the interest shown by Mr. R. W. Marsh of Long Ashton Research Station and Mr. L. Ogilvie of the N.A.A.S. and members of his staff, during the course of the investigation and the assistance of Miss M. Marlow and Miss P. Tozer in recording the results.

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THE CONTROL OF FERMENTATION IN CIDER. I. THE INFLUENCE OF NITROGEN AND ACCESSORY GROWTH FACTORS ON THE STABILITY OF CENTRIFUGED CIDERS.

By L. F. BURROUGHS.

Introduction.

In previous papers (1, 2, 3 and 4) the fermentations of two Bramley's Seedling juices have been examined. The course of yeast growth and the distribution of yeast, in the lees and in suspension, were traced by following the changes in the total- and soluble-nitrogen contents of the fermenting juices. The study of these undisturbed fermentations (1 and 3), which were allowed to proceed to completion, was combined with an investigation of the principles underlying the use of the centrifuge to check the fermentation of cider (2 and 4). Replicate casks of the same juices were centrifuged at different times during fermentation and the resulting ciders were stored in casks for observation of their stability.

In these experiments, the stability of a cider is regarded as its resistance to further fermentation by yeast and is measured by its ability to maintain its specific gravity during storage. Complete stability in this sense is only rarely encountered in cider-making and, in fact, although fermentation of these ciders was checked by centrifuging, all showed some further loss of specific gravity. With one of these Bramley juices it was found (4) that the relative stability of the ciders was related to the extent of yeast growth prior to centrifuging and that further yeast growth and fermentation in the centrifuged ciders were probably limited by a deficiency of one or more of the accessory growth factors rather than of nitrogen.

The present paper is a continuation of this investigation, again using a Bramley's Seedling juice. A similar experimental procedure has been followed, namely : (a) a detailed examination of the undisturbed fermentation ; (b) observation of the stability of ciders centrifuged at different specific gravities ; and (c) an investigation of the nutritional deficiencies limiting further yeast growth and fermentation in one of the centrifuged ciders.

Experimental.

Processing and Sampling Procedure.

Approximately 3½ tons of Bramley's Seedling apples were washed, milled and pressed on 18/12/50 yielding about 600 gallons of juice which was bulked in a slate vat. After very thorough mixing, the juice was pumped uniformly into seven hogsheads. Meanwhile a portion of the bulked juice was pumped through the Sharples centrifuge at 16,000 revs./min. and 80 gal./hour, collected in a Prodorglas-lined tank and finally filled into another hogshead. This same centrifuging procedure was used throughout this experiment. After filling with juice, all casks were kept in the Cider-house and fermentation was allowed to proceed normally.

The juice in Cask 1 was allowed to ferment to dryness without disturbance

and was not centrifuged at any time. The centrifuged fresh juice in Cask 2 was centrifuged a second time when its specific gravity was 1,020. The fermenting juice in each of Casks 3 to 8 was centrifuged at different stages of fermentation, i.e. at progressively decreasing specific gravities. Each cider after centrifuging was stored in a 40-gallon cask. The cider from Cask 3, centrifuged at specific gravity 1,034·5, was centrifuged a second time at S.G. 1,030·2 and was then stored in an 18-gallon cask.

Samples for analysis were taken from each cask as follows : (a) fresh juice : (b) at suitable intervals during fermentation prior to centrifuging ; (c) immediately after centrifuging ; (d) at suitable intervals during storage of the centrifuged cider.

Analytical Methods.

Specific Gravity was determined at 20° C. using specific gravity bottles. Weighings were made against a counterpoise as described by Siebel and Kott (5).

Sugars were determined by Somogyi's reagent (6) ; details of the procedure have been given in an earlier paper (3).

Nitrogen was determined on samples which had been acidified with 1/10th volume of 11N H_2SO_4 and stored in 4 oz. bottles. The micro-Kjeldahl method used for nitrogen determinations was also described in the reference quoted above (3).

Phosphorus was determined on other portions of the same samples used for nitrogen determinations. The wet-digestion procedure with perchloric and nitric acids, as previously described (3), was again used but phosphorus was determined on the whole digest by the method of Hanson (7) using a combined molybdate-vanadate reagent. Duplicate determinations were made.

Samples of juice or cider for determination of soluble nitrogen and soluble phosphorus were filtered, using kieselguhr and a sintered glass funnel, to remove suspended solids. In contrast, samples for total nitrogen and total phosphorus were not filtered and these determinations therefore include nitrogen or phosphorus in suspension. The kieselguhr (" Hyflo ") used was previously extracted with acid to remove any nitrogen or phosphorus compounds which might otherwise contaminate the samples being filtered. Tests on the treated material showed that it neither adsorbed nor liberated soluble nitrogen or phosphorus.

Results.

The composition of the bulked juice was as follows : Specific Gravity, 1,043·8 ; total reducing sugars after inversion, 9·35 g./100 ml. ; titratable acidity (as malic), 0·83 g./100 ml. ; pH, 3·20 ; " tannin," 0·08 g./100 ml. ; rate of fermentation at 25° C., 4·0 ; total- and soluble-nitrogen, 9·02 and 8·54 mg.N 100 ml. respectively ; total- and soluble-phosphorus, 4·97 and 4·93 mg.P/100 ml. respectively.

The Undisturbed Fermentation.

The juice in Cask 1 was allowed to ferment to dryness and was left to stand on its lees for autolysis of yeast to take place.

The change in specific gravity and sugar content of the juice during fermentation are shown in Fig. 1.

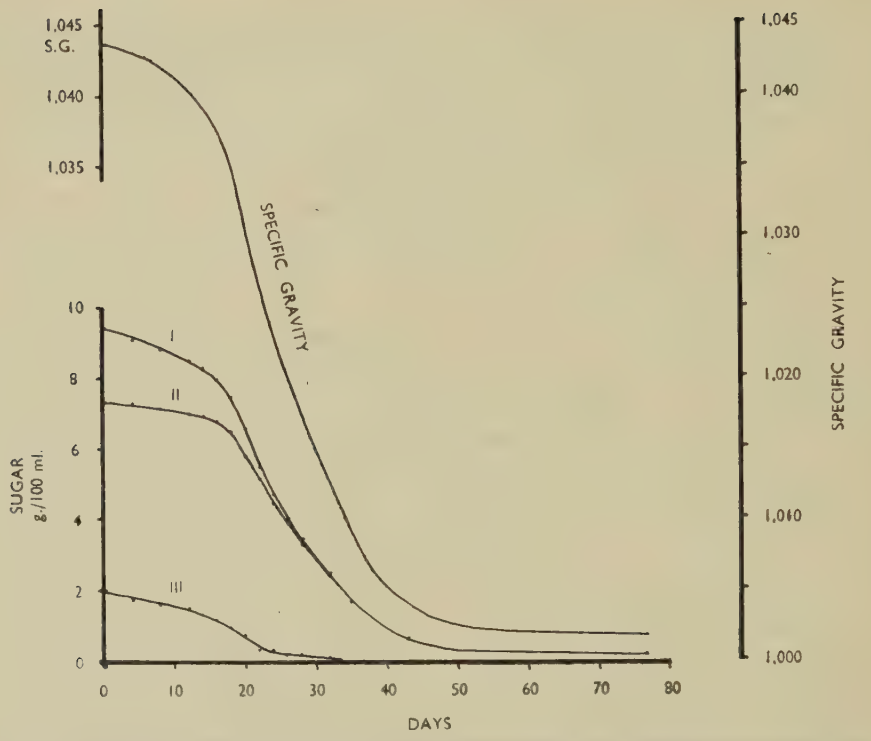

Fig. 1. Changes in Specific Gravity and Sugar Content during the Undisturbed Fermentation.
Curve I. Total reducing sugars after inversion (as invert sugar).
,, II. Reducing sugars before inversion (as invert sugar).
,, III. Sucrose.

Fermentation was slow during the first ten days, then gradually became more rapid and was at its maximum rate (averaging 2·0 degrees S.G. per day) between the 18th and 25th days. The rate decreased appreciably between the 25th and 37th days (averaging 1·16 degrees S.G. per day) after which fermentation became much slower and had practically ceased by the 50th day. The specific gravity remained constant at 1,001·8 between the 77th and 170th days but decreased to 1,000 at the 278th day. This decrease, which is indicated in the specific gravity curve in Fig. 2, could not be accounted for by the disappearance of sugar. It was due to a malo-lactic fermentation since the titratable acidity of the cider decreased from 0·86 to 0·48 g./100 ml. during this time ; a malo-lactic bacterium was in fact isolated from this cider.

The curve showing the decrease in total sugar content followed a course similar to that for specific gravity and requires no further comment. Reducing sugars showed only a small decrease during the first 18 days, due to the inversion of sucrose, which by the 25th day was almost complete.

Changes in Nitrogen Content during Fermentation.

The significance of the terms soluble nitrogen and total nitrogen and their use in calculating data representative of the growth and distribution of yeast, have been explained in a previous paper (1).

The nitrogen data for Cask 1 is shown in Fig. 2 simply as the changes in total- and soluble-nitrogen, with the derived curve for nitrogen in suspension. A brief interpretation of these curves in terms of yeast growth is given below, but no detailed comparisons will be made between yeast growth in this juice and in those previously described.

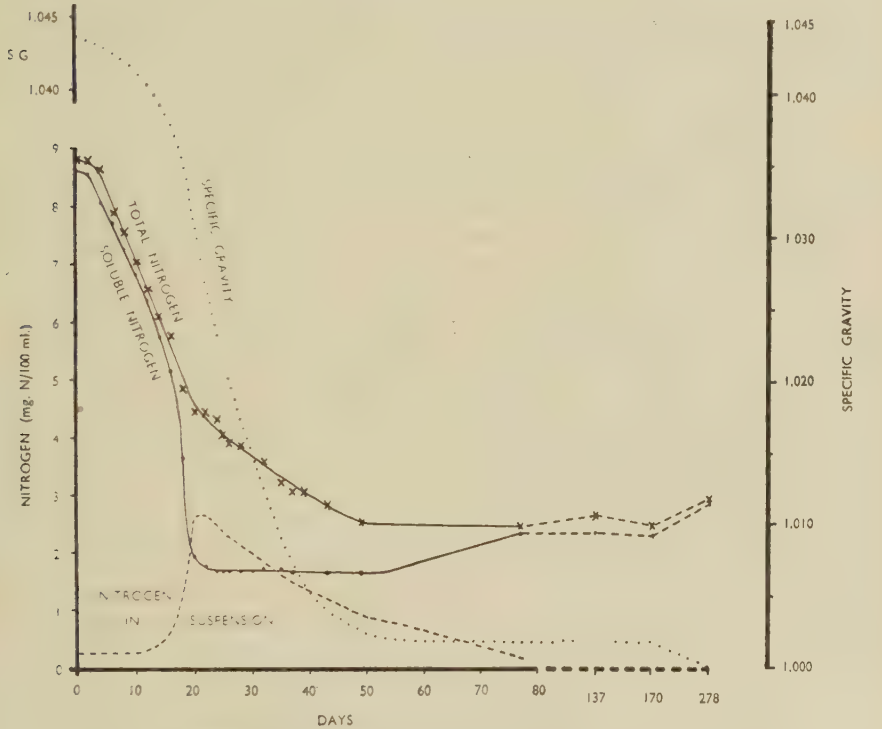

Fig. 2. Changes in Nitrogen Content during the Undisturbed Fermentation.

After a short lag period of two days, yeast growth took place at a steady rate until about the 15th day, but there was no increase in the amount of yeast in suspension. During this time fermentation was relatively slow, possibly due to the rather low temperatures which prevailed—the daily average temperature* was in the region of 42° F. After about the 16th day, rate of yeast growth, yeast in suspension and rate of fermentation all increased. This coincided with a rise of about 5° F. in the daily average temperature which may have contributed to these effects although it is not possible to say to what extent.

Yeast growth ceased about the 23rd day when the specific gravity was approximately 1,025 and soluble nitrogen 1·70 mg.N/100 ml. Yeast in suspension was then at its maximum, representing 2·65 mg.N/100 ml., and the ratio of yeast in suspension to that in the lees was 3:5. After this date, yeast in suspension decreased rapidly.

There was a marked increase in soluble nitrogen between the 50th and 77th days, due to autolysis of yeast. No further autolysis was detected until

* The average of daily maximum and minimum temperatures in the vicinity of the casks.

the period between the fourth and sixth months during which time soluble nitrogen increased from 2·28 to 2·87 mg.N/100 ml. The total increase in soluble nitrogen due to autolysis represented 17% of the amount of soluble nitrogen utilised for yeast growth.

Changes in Phosphorus Content during Fermentation.

The changes in total- and soluble-phosphorus, shown in Fig. 3, followed an essentially similar course to those for total- and soluble-nitrogen.

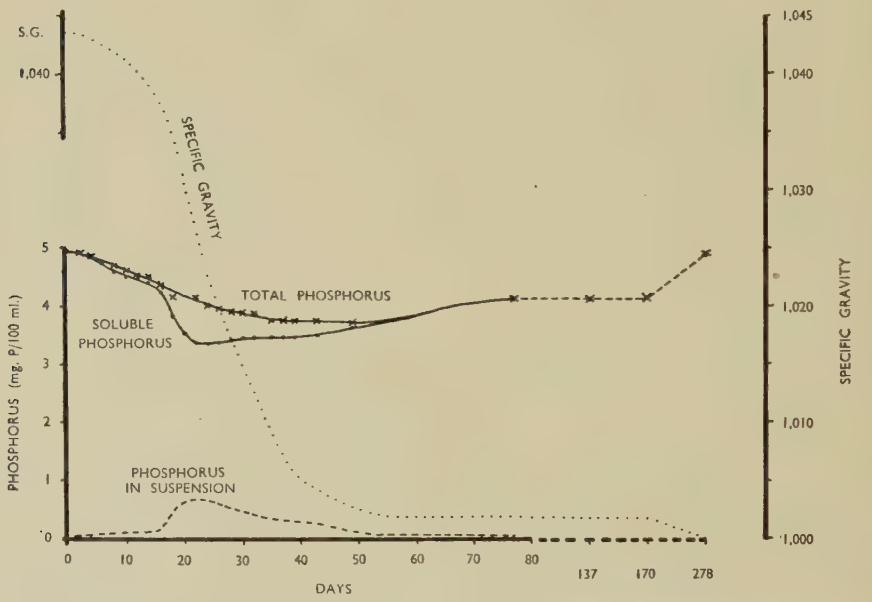

Fig. 3. Changes in Phosphorus Content during the Undisturbed Fermentation.

The amount of soluble phosphorus utilised up to the 23rd day, when yeast growth apparently ceased, was 1·59 mg.P/100 ml.; the corresponding amount of soluble nitrogen utilised was 6·90 mg.N/100 ml. The ratio in which nitrogen and phosphorus were utilised for yeast growth was therefore 4·35:1 which is in fair agreement with the N/P ratio of the yeast in suspension at the 23rd day (4·50:1).

Chief interest, however, attaches to the fact that after standing on the lees for six months, the soluble phosphorus content of the cider had returned to that of the original fresh juice. This indicates that all the phosphorus utilised by the yeast during growth was liberated as a result of autolysis.

Fermentation of the Centrifuged Fresh Juice.

The main effects of centrifuging the fresh juice (Cask 2) are summarised below.

(*a*) Onset of fermentation was delayed by about fifteen days, compared with that of the untreated juice (Cask 1).

(*b*) The rate of fermentation of the juice in Cask 2 was appreciably less than that of the undisturbed fermentation in Cask 1. The maximum rate of fermentation was reached between the 32nd and 39th days and averaged 1·64 degrees S.G. per day.

(*c*) The nitrogen data indicated that yeast growth was slow during the first eighteen days but thereafter proceeded at a fairly rapid and uniform rate until the 39th day.

(*d*) There was no marked peak in the amount of nitrogen in suspension in the juice in Cask 2 such as was observed for the more rapidly fermenting juice in Cask 1.

On the 39th day, the fermenting juice in Cask 2 was centrifuged again at specific gravity 1,020 to check the fermentation. At this time the soluble nitrogen content had been reduced to 1·85 mg.N/100 ml. The subsequent behaviour and stability of this centrifuged cider during storage are described in the following section.

The Effect of Centrifuging at Different Stages during Fermentation.

The fermenting juices in Casks 3 to 8 were centrifuged at different specific gravities and the resulting ciders stored in casks for observation of their stability. Prior to centrifuging, each of these juices fermented at a rate similar to that of the juice in Cask 1, all casks showing a high degree of uniformity. Fig. 4 shows the subsequent changes in specific gravity of these centrifuged ciders up to the 278th day together with the specific gravity curves for the undisturbed fermentation (Cask 1) and for the centrifuged fresh juice in Cask 2, which was centrifuged a second time at S.G. 1,020.

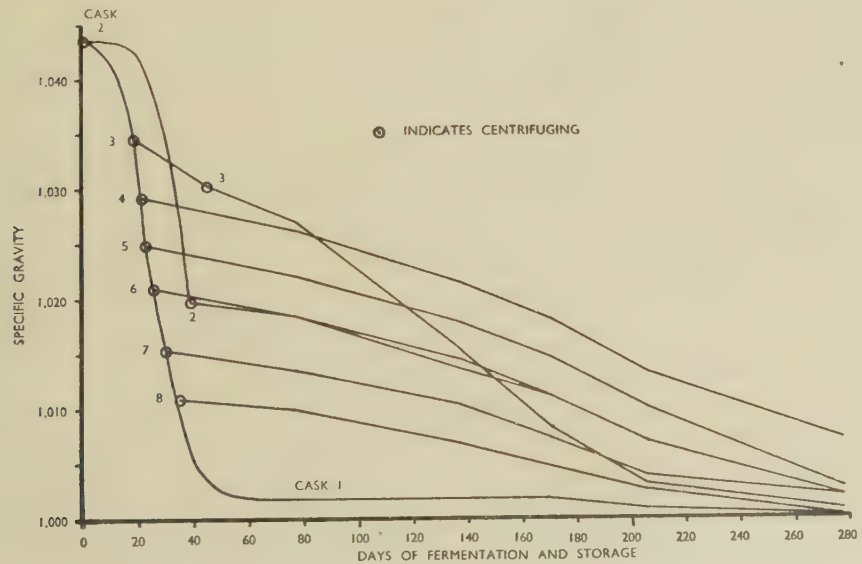

Fig. 4. Changes in Specific Gravity of Centrifuged Ciders during Fermentation and Storage.

The cider in Cask 3, centrifuged at S.G. 1,034·5 continued to ferment at the rate of about one degree per week. It was therefore centrifuged a second

time when the specific gravity had reached 1,030·2 in an attempt to obtain a more definite check of the fermentation. This second centrifuging, however, was not very effective and the rate of fermentation was only temporarily reduced, although the cider in Cask 4, which received its first and only centrifuging at S.G. 1,029·3 was relatively stable.

Apart from the cider in Cask 3, the fermentation of all other ciders may be regarded as having been more or less checked by centrifuging. However, a slow fermentation continued in these ciders during the storage throughout the summer and by the 40th week (late September) all ciders except that in Cask 4 were below 1,003 in specific gravity. It is interesting to note that the cider in Cask 2 during storage behaved very similarly to that in Cask 6. This suggests that centrifuging the fresh juice did not fundamentally affect the growth of yeast but merely delayed the onset of fermentation.

The cider in Cask 3 was also distinct from the other ciders in its soluble nitrogen content at time of centrifuging. At the first centrifuging this was 3·58 and at the second 2·11 mg.N/100 ml. ; all other ciders at time of centrifuging had soluble nitrogen contents between 1·8 and 1·9 mg.N/100 ml., except the cider in Cask 5 which was slightly lower. This indicates that yeast growth was less complete in the cider in Cask 3 than in the other ciders.

The slow fermentation which took place in all ciders after centrifuging was accompanied by further growth of yeast and at the 278th day the soluble nitrogen content of all ciders ranged between 1·04 and 1·26 mg.N/100 ml.

Investigation of the Factors Responsible for the Stability of the Ciders after Centrifuging.

In the previous paper (4) it was found that the stability of the centrifuged ciders of that experiment was probably due to a deficiency of one or more accessory growth factors. A similar experiment involving the addition of a range of yeast nutrients was therefore made on one of the centrifuged ciders of the present experiment.

The cider for this experiment was taken from Cask 4 ; its specific gravity was 1,021 and soluble nitrogen content 1·39 mg.N/100 ml. A preliminary investigation showed that the cider contained viable yeasts and no yeast inoculum was therefore added. The experimental procedure was similar to that described previously (4), the cider to which the required nutrients had been added being contained in 4 oz. medicine bottles for observation of fermentation and yeast growth. The yeast nutrients, other than the growth factors, added in the different treatments are shown in Table I. Growth factors were added as a composite solution of which 1 ml. in 110 ml. of cider provided each of the seven growth factors at approximately the concentration recommended by Hopkins and Pennington (8) for the growth of yeast. These growth factors are enumerated in Table II and have also been quoted, together with the concentrations, in the previous paper (4). When each bottle had been filled to the neck, a few drops of liquid paraffin were added to prevent growth of *Mycoderma*, the bottle was closed with a fermentation lock and was finally weighed. All bottles were kept at room temperature for yeast growth and fermentation to take place.

The bottles were weighed at suitable intervals to follow the progress of fermentation. The experiment was concluded after twenty-five days at which time some treatments were approaching dryness. Fermentation locks were

then removed, the liquid paraffin pipetted off, and the contents of each bottle thoroughly mixed to resuspend the yeast uniformly in the cider. The amount of yeast present in each cider was then determined turbidimetrically by centrifuging 10 ml. aliquots of cider and resuspending the yeast in 10 ml. of normal saline. Turbidities were measured on a Spekker photoelectric absorptiometer using neutral grey filters and were converted into percentage moist yeast from a standard curve obtained with suspensions of bakers' yeast in normal saline.

Table I shows the nutrients added in the different treatments, each in triplicate, and the responses obtained in terms of fermentation (loss in weight due to evolution of CO_2) and of yeast growth.

TABLE I.

Fermentation and Yeast Growth Resulting from the Addition of Yeast Nutrients to Centrifuged Cider (Cask 4).

Nutrient added	Fermentation. Loss in weight due to evolution of CO_2 (g./110 ml.)		Yeast Growth as percentage moist yeast (g./100 ml.)	
	No Growth Factors	Plus Growth Factors	No Growth Factors	Plus Growth Factors
No additions (Control)	0·09	0·64	0·016	0·045
	0·09	0·35	0·016	0·032
	0·07	0·42	0·016	0·032
* Minerals	0·04	0·55	0·022	0·046
	0·01	0·45	0·022	0·042
	0·04	0·38	0·022	0·038
† Nitrogen	0·09	1·91	0·050	0·284
	0·14	2·06	0·050	0·390
	0·02	1·88	0·039	0·310
Nitrogen plus Minerals	0·02	1·80	0·048	0·290
	0·01	1·62	0·055	0·246
	0·00	1·52	0·058	0·244

* Mineral nutrients consisted of *Phosphorus* as KH_2PO_4, *Magnesium* as $MgCl_2$, *Calcium* as $CaCl_2$, *Sulphur* as K_2SO_4, each element being added at the rate of 5 mg./100 ml., and *Micro-nutrients* iron, manganese, copper, zinc and molybdenum each added at the rate of 1 p.p.m.

† Nitrogen was added as NH_4Cl at the rate of 10 mg.N/100 ml.

These results show clearly that both nitrogen and growth factors were required to obtain a marked response in fermentation and yeast growth.

When only one of these was added the response obtained, although in most cases statistically significant, was much less marked. Thus, in the absence of added growth factors the addition of nitrogen increased the amount of yeast growth (significant at $P < 0{\cdot}001$) but no increase in fermentation was observed. When growth factors were added in the absence of nitrogen, both fermentation and yeast growth were significantly increased ($P < 0{\cdot}001$).

A second experiment was designed to determine which of the growth factors was concerned. The experimental technique was similar to that just described. The specific gravity of the cider (Cask 4) was 1,013·2 and the duration of the experiment was sixteen days. In one treatment nitrogen and all seven growth factors were added. In other treatments the effect of individual growth factors in the presence of nitrogen was investigated by a technique of omission, i.e. each of the seven growth factors was omitted in turn, the other six being present. The treatments, each in duplicate, and the resulting fermentation and yeast growth obtained, are shown in Table II. After five days, it became apparent that fermentation and yeast growth were taking place in all treatments except where aneurin was omitted. A further treatment was therefore put up in which aneurin was the only growth factor added; this was also continued for sixteen days to be comparable with the other treatments.

At the end of the experiment, yeast growth was measured turbidimetrically as described above and the specific gravity of each cider was determined by bottle at 20° C.; fermentation was then expressed in terms of loss of specific gravity.

TABLE II.

Fermentation and Yeast Growth in Centrifuged Cider (Cask 4) to which Nitrogen and Different Combinations of Accessory Growth Factors had been Added.

Treatments. Addition of Growth Factors	Fermentation. Loss of Specific Gravity (degrees S.G.)		Yeast Growth. Percentage moist yeast (g./100 ml.)	
No Growth Factors	1·4	1·3	0·050	0·050
Complete Growth Factors	7·7	7·5	0·260	0·260
do.—omitting Pyridoxin	8·0	10·0	0·380	0·285
,, ,, Inositol	8·1	9·4	0·255	0·385
,, ,, Ca pantothenate ..	8·7	7·4	0·280	0·235
,, ,, Nicotinic Acid ..	8·3	9·0	0·275	0·355
,, ,, Biotin	9·0	8·1	0·340	0·285
,, ,, p.Aminobenzoic Acid..	7·5	8·1	0·270	0·285
,, ,, Aneurin	3·5	3·6	0·120	0·125
Addition of Aneurin alone	6·9	7·4	0·195	0·240

Statistical treatment of the data in Table II showed that when aneurin was omitted from the added growth factors, the response obtained was significantly reduced for both fermentation ($P < 0{\cdot}01$) and yeast growth ($P < 0{\cdot}05$). No treatment involving the omission of any single growth factor other than aneurin was significantly different in its effects from the complete growth factor treatment. Furthermore, when aneurin was the only growth factor added fermentation and yeast growth were not significantly different from those obtained when all seven growth factors were added.

These two experiments clearly demonstrate that the stability of this centrifuged cider was due to deficiencies of both nitrogen and aneurin. At the same time, some stimulating effect must be attributed to the other six growth factors since when these were added in the absence of aneurin, both fermentation and yeast growth were appreciably greater than when no growth factors were added at all.

Discussion.

The utilisation of soluble nitrogen during fermentation of Bramley's Seedling juice has now been investigated in three successive years and in two of these, data for soluble phosphorus have also been obtained. The general course of the growth of yeast and of its subsequent autolysis has shown fundamental similarities in all three juices although certain differences of detail and quantitative differences in the changes involved have been observed.

In the study of the undisturbed fermentation of the present experiment, the most outstanding feature was the fact that all the soluble phosphorus utilised for yeast growth was liberated into the cider during six months standing on the lees. This indicates that the amount of phosphorus in the lees at the end of this time was negligible and, consequently, either that autolysis of the yeast was virtually complete, or that the phosphorus content of any viable cells remaining was within the limits of experimental error. The increase of soluble nitrogen due to autolysis was relatively less marked than that of soluble phosphorus. It is not possible to say to what extent these autolytic changes were influenced by the malo-lactic fermentation which occurred during the later weeks of storage.

After centrifuging, all ciders were relatively stable during storage with the exception of that in Cask 3. Consideration of the soluble nitrogen data showed that yeast growth at the time of centrifuging was less complete in the cider in Cask 3 than in the other ciders. This fact, together with the greater tendency of this cider to ferment, supports the observation made previously (4) that the stability of a centrifuged cider is related directly to the extent of yeast growth prior to centrifuging.

It was found by addition of different combinations of yeast nutrients to one of the centrifuged ciders (Cask 4) that the stability of this cider was due to deficiency both of nitrogen and of aneurin. Although this was the only cider examined in this way, it is reasonable to suppose that the same deficiencies were operative, to at least the same extent, in ciders centrifuged at later stages in the fermentation.

With the information at present available it is not possible to draw any general conclusions regarding the influence of aneurin in cider fermentations. The growth factor experiments described in this paper were concerned with the relation between the nutritional status of the centrifuged cider and the

micro-flora which it contained. A microbiological investigation is now needed of the nutritional requirements of the various micro-organisms present in cider during fermentation and also after centrifuging.

Work is at present in progress with other centrifuged ciders to ascertain the factors responsible for their stability. Details of this will be given in a subsequent paper but preliminary results suggest that in at least three instances stability is again due to a combined deficiency of nitrogen and aneurin. It is, however, possible that in certain ciders other deficiencies may arise, depending on the composition of the juice and on the nutritional requirements of the micro-organisms present.

Summary.

(1) The changes in nitrogen and phosphorus content of a Bramley's Seedling juice have been examined during fermentation and during storage of the cider.

(2) Other portions of the same juice were centrifuged at different stages of fermentation and observations made on the stability of the resulting ciders during storage.

(3) Different combinations of yeast nutrients were added to one of the stable ciders and its stability was shown to be due to deficiency of both nitrogen and aneurin.

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THE USE OF SELECTED YEASTS IN CIDER-MAKING. PROGRESS REPORT I.

By F. W. BEECH, L. F. BURROUGHS and R. C. CODNER.

Introduction.

In the early production of fermented beverages, such as beer, wine and cider, the fermentations were carried out by the use of unknown mixtures of yeasts and bacteria found naturally on the raw materials. Following the work of Pasteur and more recently of Hansen, the use of specially selected yeasts was gradually introduced into the beer and wine industries with the object of achieving a greater degree of control of the fermentation and thereby assisting in the production of more standardised and improved products. Addition of either a pure strain of yeast or a mixture of yeast strains of known origin to the wort or must is now the widespread practice in the manufacture of these two products (1, 2).

The use of pure yeast cultures for cider-making has been common practice in Germany for many years (3) and more recently in Switzerland (4). In these two countries, apples used for cider-making are of relatively high acidity and the ciders produced by natural fermentation are rather thin by English standards. When selected yeasts are used for the fermentation, the ciders produced take on a more vinous aroma and flavour and show some resemblance to light, dry, white wines.

In England and France the majority of the apples used for cider-making possess a certain amount of tannin which gives a characteristic flavour to the fermented drink. The juices are allowed to ferment naturally, producing ciders which are entirely different from those of Germany and Switzerland. For example, " body " and apple character are rated highly and in France particularly, a full-bodied, bittersweet cider is required (4).

In England, attempts at fermentation with selected yeasts, starting with the work of Lloyd at Butleigh Court, have shown that the flavour of the resulting cider was influenced both by the character of the fruit and by characters derived from the yeast (5). The early work was concerned mainly with the assessment of changes in aroma and flavour (6) rather than with the degree of dominance attained. Thus little evidence could be brought forward to show the extent to which the added yeasts were responsible for the fermentations that ensued. The problem of identifying selected yeasts present in a mixed flora is extremely difficult and it is only now, in the light of modern work on yeast nutrition, that the problem can be tackled with any degree of confidence.

Apart from the scientific aspects of the subject there are at present two opposing viewpoints concerning the practical use of selected yeasts in English cider-making. Some believe that the general use of pure yeast cultures would bring about an improvement in the cider produced (7). Against this there is a widely held opinion that a typical " English " cider can only be produced by the mixed microflora found in a " natural " fermentation and that if a selected yeast is used the result is an apple-wine (8). It may be that the protagonists of selected yeasts are more used to working with fruit similar

to that found in Germany and Switzerland whereas those who hold the opposite view are probably more familiar with West Country cider apples. However it must be admitted that for certain special types of cider a pure yeast culture is essential, e.g. a flocculating, slow fermenting yeast for making champagne cider. Irrespective of this divergence of opinion on the desirability of selected yeast fermentations, a study of such fermentations and of the factors influencing dominance and yeast succession should provide valuable information on cider fermentations in general.

The use of selected yeasts in fermentation can be classified under :—

(i) Pure Culture Methods—where a selected yeast is added to a sterilised juice.

(ii) Dominant Fermentation—where the juice is not sterilised but where the conditions are made such that the selected yeast can develop preferentially. This yeast can either be added as a culture or be a yeast or strains of a species of yeast actually present in the juice.

In the 1949-50 and 1950-51 cider-making seasons, both methods (i) and (ii) were investigated and a summary of these results is given in this Report.

Experimental.

The yeasts and juice treatments used in the two season's work were as follows :—

Origin of the Selected Yeasts.

The selection of the yeasts used in this work was based on results of preliminary trials by Barker (6) involving some seventy-five strains. The yeasts chosen are classified below :—

Cider Yeasts.

T.B.6, K.B.SS3, T.5, M.B.2 ; isolated by Professor B. T. P. Barker from Kingston Black juice and ciders.

Wine Yeasts.

Fendant and Herrliberg ; yeasts widely used in the Swiss cider-making industry and supplied by courtesy of Dr. Lüthi, Research Station, Wädenswil.

Champagne ; isolated from champagne by Mr. H. J. Bunker.

Jeres ; a sherry yeast.

Production of the Starters.

The stock yeast cultures at the Research Station are maintained as streaks on agar slopes. This medium contains 3% Malt Extract, 1% Difco Yeast extract, 1% glucose and 2% agar.

The inoculum was increased in quantity by transferring a loopful from the appropriate slope to 10 ml. of fortified apple juice. This was prepared by adding the following to apple juice :—1% Marmite, 0·1% di-ammonium hydrogen phosphate and 0·2% magnesium sulphate. The medium, sterilised by autoclaving, was used for all stages of starter production except the final 2½ gallon starter for which boiled, unfortified apple juice was used. The yeast cultures were increased from an initial 20 ml. (2 test tubes) through 100 ml.

and 1,500 ml. stages to the final 2½ gallon starters. Each culture was incubated at 25° C. for 24 to 48 hours to allow active fermentation to set in before transferring to the next stage.

As sulphur dioxide (SO_2) was to be added to one series of fermentations, the selected yeasts for this series were acclimatised to SO_2 on a test tube scale by culturing the yeasts successively in fortified apple juice containing 12½, 100 and 150 p.p.m. SO_2 until active fermentation set in at each level. After the acclimatisation stage, the juice for the remaining stages of starter production for this group of yeasts contained 150 p.p.m. SO_2.

Juice Treatments.

Before the main experiments were begun it was necessary to gain preliminary information on suitable methods for restricting the action of the natural juice flora. Various treatments were applied singly or in combination to one-gallon portions of a uniform bulk of juice from the variety Coleman's Seedling. The times taken for the juice to ferment spontaneously after such treatments are summarised below in Table I.

TABLE I

The Delaying Effect of Different Juice Treatments on the Onset of Natural Fermentation.

Juice Treatments	Time Taken for Active Fermentation to Commence
1. Control (Fresh juice, untreated)	4 days
2. Fining and Racking	9 days
3. Centrifugation	9 „
4. Addition of 100 p.p.m. SO_2	9 „
5. Filtration with a coarse filter-aid	9 „
6. Fining, Racking and Centrifugation	12 days
7. Fining, Racking and Addition of 200 p.p.m. SO_2	1 month
8. Centrifugation and Addition of 200 p.p.m. SO_2	1 „
9. Addition of 200 p.p.m. SO_2	1 „
10. Enzymic depectinisation and Pulp-filtration	3 months *
11. Enzymic depectinisation and Sheet-filtration (K5 sheets)	3 „ *
12. Fining, Racking and Pulp-filtration	3 „ *

* These juices were remarkably stable; their specific gravity decreased by only 10 degrees during 12 months.

These results strictly only apply to the particular juice used and it cannot be said that any other juice subjected to the same treatments will react in exactly the same way. However, the experiments do show the type of effect to be expected with other juices.

It was considered that a treatment giving inhibition of the natural flora for a short time would permit the selected yeasts to achieve dominance. For this reason the addition of 100 p.p.m. SO_2 was used.

General Cider-Making Procedure.

In ordinary cider-making it is customary to blend different ciders together in order to produce a beverage which is balanced with respect to sugar, acid and tannin. In the present work, however, it was desired to compare the ciders produced from the same original juice. This made it necessary to use an initial blend of juice such that the ciders produced would be suitably balanced in flavour. True cider varieties were used in both seasons and the blends were similar in acid and tannin content.

In both seasons, the fruit was washed, milled and pressed, only the first pressings being used. The juice was bulked, mixed thoroughly and then divided amongst several smaller vats in which appropriate juice treatments were carried out. The treated juices were distributed between freshly steamed 120-gallon fermentation barrels, each of which contained $2\frac{1}{2}$ gallons of the appropriate starter. Unyeasted controls were also prepared for each juice treatment. For the sulphited juice treatment in the first season's test, 100 p.p.m. SO_2 was added to the fresh juice but as the unyeasted control began to ferment after only six days, the delay was considered to be insufficient. In the next season, therefore, the amount of SO_2 added was increased to 150 p.p.m. giving a delay of 11 days before the unyeasted, sulphited juice commenced fermentation. In each season the sulphited juice was allowed to stand overnight after the addition of SO_2 and was racked into the barrels next morning.

For pure culture fermentations it was necessary to remove the natural flora completely. To this end the juice was first treated overnight with a pectin-destroying enzyme (0·2% Pectozyme). Next day when a test on a sample of the treated juice indicated that the pectin was completely degraded, the juice was pulp- and sterile-filtered into steamed barrels containing the appropriate starters.

During fermentation, the heads were allowed to work out of the barrels and air locks were then fitted. At S.G. 1,025 the ciders were centrifuged; in the first season these ciders were left in sealed 60-gallon casks for approximately two months to mature, after which they were filtered through K5 filter sheets, bottled and allowed to condition naturally. In the second season, all the ciders were filtered and bottled for natural conditioning immediately after centrifuging. In addition, samples from some of the centrifuged ciders were clarified with K5 filter sheets, carbonated artificially, sterile-filtered and bottled in sterile bottles. This was carried out immediately after centrifuging and also after storing in filled and sealed glass jars for ten weeks.

Analytical Methods.

The following determinations were made on samples of the fermenting juices and ciders at suitable intervals, using the methods already reported (9) :—

Specific Gravity : Titratable Acidity : Volatile Acidity : pH : "Tannin" : Sulphur dioxide : Nitrogen : Total Reducing Sugars. The Alcohol contents of these ciders were determined from the specific gravity of the distillates and expressed as ethyl alcohol, g./100 ml. (10).

Microbiological Methods.

In view of the exploratory nature of the two season's work it was decided to differentiate the yeasts present in the fermenting juice solely on morphological characteristics. Initially, samples of the fermenting juices were compared microscopically with the appropriate culture yeast as direct wet preparations. It was found, however, to be almost impossible to form an estimate of dominance because of the variation in cell form (pleomorphism) exhibited by the yeasts during fermentation.

It was decided therefore to compare microscopically the smears from twelve colonies obtained by plating out a known volume of a sample on the agar medium* with a smear from a colony of the appropriate selected yeast that had been grown under the same conditions. By this means confusion due to pleomorphism could be eliminated. The proportion of colonies containing yeasts resembling those from the colony of the selected yeast gave an estimate of the degree of dominance attained. For some of the selected yeasts additional evidence was obtained from the macroscopic appearance of the colonies.

Results.

In this work, portions of a uniform bulk of juice were submitted to several juice treatments and inoculated with selected yeasts. The ciders produced from these yeasted juices showed varying degrees of difference in chemical composition, aroma and flavour when compared with their controls. Before it can be said that these differences were due to the action of the yeasts added originally to the juice it is essential to show that the yeasts were in fact dominant during fermentation. Direct evidence of dominance during a fermentation can only be given by microbiological observations, which should establish the identity of the yeast present in the greatest numbers in a sample with that of the selected yeasts added originally. When microbiological data indicate that the added yeast is dominant then differences observed in fermentation characteristics, chemical composition, aroma and flavour can be attributed to the selected yeasts. Furthermore, the ciders should be tasted under strictly controlled conditions and the results submitted to statistical analysis. In this preliminary work it was not possible to fulfil these requirements completely for reasons which are outlined in the Discussion.

Microbiological Observations.

An attempt was made to obtain some estimate of the proportion of the total yeast *in suspension* contributed by the selected yeast. When this was greater than 50%, the selected yeast was regarded as dominant. The lees were not examined at any stage for the presence of selected yeasts. The limitations of this method are discussed later.

Within the limits of this technique, it was found in the first season's work

* See "Production of Starters."

that all the selected yeasts added to sulphited juice were dominant during fermentation to S.G. 1,025. The ciders were then centrifuged, stored for two months, K5 filtered and bottled. In the filtered ciders the proportion of selected yeasts present had decreased considerably, and in many, the added yeasts were no longer present. Similar results were also obtained from an examination of the deposits in these ciders after natural conditioning for seven weeks in the bottle. It is obvious, therefore, that the treatment given between S.G. 1,025 and bottling had profoundly altered the balance of micro-organisms present in the fermenting juice and it is doubtful if the ciders had been conditioned by the selected yeasts originally added.

In the second season, two juice treatments were examined, namely (a) sterile-filtered juice, and (b) juice treated with sulphur dioxide. In the first series (a), the selected yeasts remained in pure culture throughout. In the second series (b), only three yeasts (T.B.6, Fendant, and M.B.2) were always dominant. The remaining four yeasts were only found to be dominant at one or more stages of examination, but as two of these yeasts flocculated strongly the microbiological examination may have given an insufficient estimate of dominance. In the same season the microbiological examination at time of bottling was restricted to the artificially carbonated and sterile-filtered ciders which were merely tested for sterility.

Fermentation Characteristics.

In both seasons the time-data have been calculated from the dates on which the yeasts were added to the treated juices.

Onset of Fermentation.

The times for onset of fermentation are given in Table II.

TABLE II

The Effects of Yeast and Juice Treatment on the Onset of Fermentation.

Season	Juice Treatment	Time Taken for Fermentation to Commence	
		Yeasted Juices	Unyeasted Treatment Control
1949-50	Addition of 100 p.p.m. SO_2	3 days—all seven yeasts	6 days
	Sterile-filtration	1 day —6 yeasts 3 days—T.5	11 days*
1950-51	Addition of 150 p.p.m. SO_2	5 days—T.B.6 6 ,, —T.5, Fendant 9 ,, —K.B.SS3 10 ,, —Champagne 11 ,, —Herrliberg, M.B.2	11 days

* Fermentation due to non-sterility of wooden barrel.

Thus, with the exception of four yeasts in the last group, juices inoculated with selected yeasts began fermentation well in advance of the appropriate controls, and therefore the desired partial inhibition of the natural flora had been achieved. Although attempts to acclimatise the four yeasts to SO_2 had not been entirely successful, one of them, M.B.2, was in fact found to be dominant during fermentation.

Rate of Fermentation.

The rates of fermentation in barrel for some of the inoculated juices between time of inoculation and S.G. 1,025 are given in Table III.

TABLE III.

Rates of Fermentation of Juices Inoculated with Selected Yeasts.

Fresh Juice to S.G. 1,025.

(All results expressed as degrees of S.G. lost per day).

Season	Juice Treatment	Rate of Fermentation				
		Treatment Control	T.B.6	K.B.SS3	Fendant	Champagne
1949-50	Addition of 100 p.p.m. SO_2 ..	0·93	1·28	1·28	1·15	0·69
1950-51	Sterile-filtration	0·39	0·96	0·69	0·62	0·29
	Addition of 150 p.p.m. SO_2 ..	0·77	1·09	0·73	1·36	0·086

It would appear that where the microbiological observations indicated that the added yeast was dominant then the same pattern of fermentation behaviour was observed each season. Thus, T.B.6, K.B.SS3, and Fendant fermented faster then their treatment controls whereas Champagne always fermented more slowly. These characteristics have also been observed in laboratory pure culture fermentations where the slow rate of fermentation of the Champagne yeast has been outstanding.

Chemical Observations.

*Nitrogen Content.**

In the first season the suspended nitrogen in the unyeasted, treatment control juice was 0·90 mg. The actual amount of yeast present in the freshly inoculated juices was not determined directly but was calculated from the volumes and nitrogen contents of the inocula and juices respectively and expressed as nitrogen in suspension. This was uniform (1·35 mg.) for all selected yeasts except T.B.6 (1·20 mg.). Thus the amounts of suspended nitrogen which could be regarded as representative of the amount of the selected yeasts added were, therefore, 0·45 mg. for all yeasts except T.B.6 which was 0·30 mg. This is remarkable since no attempt was made to standardise the amounts of yeast added.

* The terms total nitrogen, soluble nitrogen, etc., have been used as defined by Challinor and Burroughs (11). All nitrogen data have been expressed as mg.N/100 ml. of juice or cider and have been abbreviated to mg. in the text.

In the second season the nitrogen in suspension was determined directly on samples of the freshly inoculated juices. This time the nitrogen in suspension, representative of the yeast added, varied from 0·38 mg. to 0·83 mg. This variation may have been due either to the differences in yeast growth during the build-up of the inocula or possibly to the samples of inoculated juice not being uniform with respect to the material in suspension. The method of estimation used in the first season would seem to be more satisfactory since uniform samples can be obtained more simply.

Using the methods of Challinor and Burroughs (11) it was possible to obtain an estimate of the growth and distribution of yeast at S.G. 1,025. Typical results for three of the selected yeasts used in the first season are shown in Table IV.

TABLE IV.

Nitrogen Data representing Growth and Distribution of Yeast at Specific Gravity 1,025.

(All results, except Yeast Distribution Ratios, expressed as mg.N/100 ml.).

		T.B.6	K.B.SS3	Champagne
Freshly Inoculated Juice	Total Nitrogen	7·30	7·30	7·30
	Soluble Nitrogen	6·10	5·95	5·95
Fermenting Juice at S.G. 1,025	Total Nitrogen	6·30	2·35	3·20
	Soluble Nitrogen	1·65	2·00	1·85
	Nitrogen in suspension ..	4·65	0·35	1·35
	Nitrogen in the lees ..	1·00	4·95	4·10
	Yeast Distribution Ratio* ..	4·65	0·07	0·33
	Yeast Growth	4·45	3·95	4·10

* The Yeast Distribution Ratio is given by Nitrogen in suspension divided by Nitrogen in the lees.

Yeast T.B.6 was very different from any of the other selected yeasts since it showed the greatest amount of yeast growth, the greatest amount of yeast in suspension and therefore the largest yeast distribution ratio, i.e. it was non-flocculating. In contrast, K.B.SS3 had the extremely low yeast distribution ratio of a flocculating yeast. The distribution ratio of Champagne yeast was also low indicating that this yeast also flocculated. The flocculation characteristics of these two yeasts were clearly seen visually.

In the second season the distribution of nitrogen in the yeasted, sterile-filtered juices was again examined at S.G. 1,025 and although these three yeasts showed the same flocculation characteristics the indications were not so definite since there was much less suspended yeast in all the fermenting juices of this series.

Efficiency of Conversion of Sugar to Alcohol.

The ratio of the amount of alcohol produced to the amount of total reducing sugars consumed gives a measure of the efficiency of this conversion by the micro-flora during any particular fermentation. This ratio was determined for the first season's ciders prepared from sulphited juice and found to range from 0·46 to 0·49 for the selected yeast fermentations, with an average of 0·47. (Niehaus (12) states that a ratio of 0·47 is usually obtained in wine-making). The ratio for the unyeasted control was 0·44, so that the addition of a selected yeast gave in these tests a more efficient conversion than the natural flora of the corresponding unyeasted juice. The same results were found in the second season's experiments for the sterile-filtered ciders at time of bottling.

Vintage Quality of the Ciders.

The vintage quality of the ciders was assessed by a small panel consisting of five members of the Cider Section. The sampling procedure has been described in earlier Annual Reports (13, 14).

Although the first season's ciders were tasted on three separate occasions only the first tastings will be discussed since the prolonged natural conditioning may have obscured any effects of the selected yeast in later tastings. The control cider was very attractive and in fact only one yeasted cider was given a higher figure of merit. The remainder were of average quality, while a few were rather poor, possessing a "yeasty" taint. K.B.SS3 produced a very pronounced "ester" aroma and flavour but apart from this no cider had an obvious flavour attributable to the yeast originally added.

In the second season, the naturally conditioned ciders were tasted approximately three months after bottling when it was found that three out of the seven yeasts produced ciders as good or better than the controls. The remainder were thin and mediocre. Sulphited juice fermented with K.B.SS3 again had an ester aroma.

In the second season, other samples of the Champagne-yeasted ciders, their corresponding treatment controls and the true control were artificially carbonated, sterile-filtered and bottled directly after centrifuging at S.G. 1,025. Further samples were similarly bottled after the centrifuged ciders had matured in glass jars for 10 weeks. It was found that the cider made from Champagne-yeasted, sulphited juice was preferable to all the other ciders in this group, either with or without maturing in glass.

Discussion.

In this series of experiments selected yeasts were added to juices treated in several ways. An examination was made of the yeasts present during fermentation to S.G. 1,025. On the basis of morphological similarities between these yeasts and the selected yeasts originally added it was found that dominance had been established in nearly all the ciders. In such ciders the following were observed :—

(a) Each of the selected yeast ciders differed from the control cider in the onset and in the rate of fermentation.

(b) At S.G. 1,025 the amount of yeast growth which had occurred in each of the fermenting juices was approximately the same, but the nitrogen distribution ratios (nitrogen in suspension ÷ nitrogen in the lees) of the

selected yeasts varied widely, e.g. in the first season the ratios varied between 4·65 to 0·07 depending on the degree of flocculation of the yeasts.

(c) All the selected yeasts appeared to be more efficient than the natural flora in their conversion of sugar to alcohol.

Thus, it appears from microbiological evidence that the majority of the selected yeasts established dominance during the primary fermentation. The resulting ciders differed in fermentation characteristics from the control ciders and there were slight differences in chemical composition. Only one cider (K.B.SS3 in SO_2– treated juice) had a characteristic flavour attributable to the selected yeast; all others differed only slightly from the controls in flavour, even where the selected yeasts had been dominant.

This work, although necessarily of a preliminary nature, has demonstrated the need for a more fundamental investigation of the whole problem of the use of selected yeasts. A study of dominant fermentations under practical cider-making conditions, as outlined in the Introduction, raises a wide range of microbiological problems, since the selected yeast must be identified in samples still containing much of the original flora. Under these conditions, to determine with certainty that the selected yeast has dominated the fermentation, it would be necessary (a) to isolate each yeast present during the fermentation, (b) to assess the proportion each yeast forms of the *total* yeasts present, (c) to determine the fermentative power of each yeast (expressed numerically, possibly as a rate of fermentation) and finally, (d) to prove the identity between the selected yeast originally added and the yeast having the greatest influence on the fermentation (e.g. having the greatest value for (b) $\times$ (c)). From the research point of view, the investigation of the use of selected yeasts would be simplified by confining the studies to pure culture fermentations. Thus, the juice can be treated either (i) so that only a selected group of yeasts remains in the juice or alternatively (ii) so that the natural flora is completely suppressed and the selected yeasts added as pure cultures. Under (i) might come the addition of sulphur dioxide, while for (ii) sterile-filtration or flash-pasteurisation can be used. Following either method it would only be necessary to establish microbiologically that the yeasts present were respectively those required as in (i) or those that were added as in (ii). Once these studies have revealed the effects of different selected yeasts then consideration can be given to their use in practical cider-making.

The effect of the selected yeasts on the flavour of ciders produced is one of the most controversial aspects of this subject. In the first place flavour differences have, in the past, been attributed to the action of selected yeasts without definite proof of dominance. Furthermore, the method of natural conditioning usually adopted may have imposed characters not due to the selected yeast. The effects on cider quality of the juice treatments required in a selected yeast fermentation are further complicating factors. In future tests it is intended to obtain a better separation of the effects of both yeast and juice treatments and to submit the tasting panel results to statistical analysis.

In order to use selected yeasts in cider-making to full advantage it is important to find out under what conditions they produce their greatest effect. Stopping the fermentations at S.G. 1,025 gives the selected yeast little opportunity for maximum flavour production. On the other hand, in these tests, when the dry ciders were allowed to stand on their lees, as quoted

for German practice (15) objectionable flavours were apparent. This was observed during the second season with fermentations of yeasted, sterile-filtered juice. In the present season the fermentations are being stopped at S.G. 1,005 in an attempt to effect a compromise.

Apart from the possible effects of selected yeasts on cider quality, a study of the effects of juice treatments and of pure culture fermentations should lead to a wider understanding of normal cider fermentations.

Summary.

(1) Investigations of cider fermentations by the use of selected yeasts have been made during two seasons.

(2) Suppression of the natural juice flora by sterile-filtration or by treatment with sulphur dioxide was used to assist the added yeasts to attain dominance.

(3) Microbiological observations of the yeasts in suspension showed that, with few exceptions, each of the selected yeasts achieved dominance during the primary fermentation.

(4) These ciders, in which dominance was demonstrated microbiologically, differed from their controls in fermentation characteristics and in their chemical composition. These differences were attributed to yeast action.

(5) The ciders produced by these fermentations were tasted and some differences in aroma and flavour were observed.

(6) Proposals for future work on the use of selected yeasts have been given.

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THE CONTENT OF ARSENIC, COPPER, IRON, LEAD AND ZINC IN APPLES, JUICES AND CIDERS.

By C. F. TIMBERLAKE.

Proposals for more stringent regulations of the amounts of certain metals in fruit juices and ciders have called for further information on the amounts that occur normally and as a result of contamination by spray residues or from processing equipment. During the 1950-51 season a preliminary study has been made of the content of copper, zinc, iron, lead and arsenic naturally present in unsprayed apples and in the juices and ciders prepared from them. This work is being followed by similar tests using apples which have received sprays of lead arsenate.

Experimental.

Apples of five dessert, four culinary, and nine cider varieties were washed in water and surface-dried with filter paper. The fruits were then sliced, placed in aluminium dishes lined with grease-proof paper and heated to dryness in an air oven at 85° C. The dried samples were stored in Cellophane bags until analysed.

Juices were extracted from the nine cider varieties in a small-scale cider press. Samples of known weight were taken in pyrex flasks for analysis from the main bulk of juice immediately after pressing. To facilitate analysis, the individual samples were allowed to ferment unchecked before the addition of acid in the subsequent digestion.

Ciders were prepared from the main bulk of these juices by allowing them to ferment in glass jars to a specific gravity of 1·025. After clarification by passage through an Alfa-Laval centrifuge, they were sterile-filtered and bottled in champagne pint bottles, from which samples were withdrawn for analysis.

Chemical Methods.

Destruction of organic matter was effected by wet digestion with a mixture of nitric, perchloric and sulphuric acids. Digestions were carried out in duplicate and copper and zinc were determined on the same aliquot of digested solution by the method of Holmes (1), copper as copper diethyldithiocarbamate in carbon tetrachloride and zinc as a dithizonate. Lead was also determined as a dithizonate (2), on a separate aliquot of digested solution. Iron was determined by means of o–phenanthroline after adjusting the pH to 3·5 by a method modified from that of Bandemer and Schaible (3). Arsenic was determined by the Gutzeit Test.

Summary of Results.

The analyses of the fresh dessert and culinary apples are given in Table I, and the analyses of cider apples, juices and ciders in Table II ; all figures are expressed as p.p.m. of fresh weight or of the liquid product.

Copper. The copper content of the seventeen varieties of apples studied

varied from 0·27–0·59 p.p.m. (mean 0·41 p.p.m.). In every case the copper content of the freshly expressed juice of the cider varieties was higher than that of the apples from which the juice was made. The figures for juices vary from 0·45–1·15 p.p.m. In the bottled cider, the copper content was lower than in the juice in six varieties and approximately equal in two varieties. The figures vary from 0·26–0·88 p.p.m.

Zinc. The zinc content of the apples varied from 0·21–0·76 p.p.m. (mean 0·51 p.p.m.). In the cider apples, zinc was always higher than copper, the ratio of zinc to copper varying from 1·2–2·4. In the dessert and culinary varieties, however, zinc was usually lower than copper, the zinc/copper ratio being 0·5–1·3. With two exceptions zinc was found to be higher in the freshly expressed juice than in the fruit, and ranged from 0·48–1·26 p.p.m. In cider, the zinc content was within the range 0·23–1·30 p.p.m., and was usually lower than that in juice.

Iron. The iron content of the apples varied from 1·0–2·5 p.p.m. (mean 1·8 p.p.m.). In the freshly expressed juice iron varied from 0·7–2·8 p.p.m. and in cider from 0·4–3·5 p.p.m.

TABLE I.

Metals in Culinary and Dessert Apples (as p.p.m.).

Variety	Copper	Zinc	Iron	Lead	Arsenic
Lane's Prince Albert ..	0·43	0·21	1·0	0·02	<0·04
Newton Wonder	0·44	0·36	1·8	0·08	<0·04
Early Victoria	0·44	0·39	1·4	0·07	<0·04
Grenadier	0·42	0·31	1·6	0·02	<0·04
Miller's Seedling	0·50	0·46	1·6	0·04	<0·04
Cox's Orange Pippin ..	0·55	0·54	1·6	0·07	<0·04
Laxton's Superb	0·59	0·64	2·5	0·13	<0·04
Worcester Pearmain ..	0·36	0·36	1·8	0·08	<0·04
Ellison's Orange	0·34	0·45	1·7	0·10	<0·04

TABLE II.

METALS IN CIDER APPLES, THEIR JUICES AND CIDERS (AS P.P.M.).

Variety	Copper			Zinc			Iron			Lead			Arsenic	
	Apples	Juice	Cider	Apples	Juice	Cider	Apples	Juice	Cider	Apples	Juice	Cider	Apples	Juice & Cider
Sweet Alford ..	0·33	0·86	0·31	0·46	0·64	0·32	1·9	1·2	0·4	0·16	0·03	0·04	<0·04	<0·02
Stoke Red	0·27	1·15	0·35	0·60	0·75	0·57	1·3	1·1	1·7	0·02	0·05	0·08	<0·04	<0·02
Crimson King ..	0·39	1·09	0·26	0·48	0·90	0·23	1·7	2·0	1·4	0·10	0·03	0·08	<0·04	<0·02
Kingston Black ..	0·52	1·03	0·88	0·68	0·68	0·65	2·2	1·1	2·4	0·02	0·05	0·07	<0·04	<0·02
Sweet Coppin ..	0·33	0·59	0·34	0·68	0·53	0·50	1·9	0·7	0·7	0·16	0·05	0·05	<0·04	<0·02
Yarlington Mill ..	0·38	0·45	0·54	0·64	0·48	0·72	2·5	1·5	2·3	0·15	0·03	0·07	<0·04	<0·02
Dabinett	0·33	0·65	0·29	0·62	0·83	1·30	1·7	2·8	1·1	0·08	0·08	0·04	<0·04	<0·02
Tardive Forestier ..	0·32	0·65	0·69	0·76	1·26	0·67	1·9	1·8	3·5	0·07	0·05	0·06	<0·04	<0·02

Lead. Lead was present in the apples in very small amounts, ranging from 0·02–0·16 p.p.m. (mean 0·08 p.p.m.). The highest figure found for juice and cider was 0·08 p.p.m.

Arsenic. Arsenic, although sometimes occurring in traces, was not present in apples in amounts greater than 0·04 p.p.m. (as As), or in juice and cider in amounts greater than 0·02 p.p.m. (as As).

Discussion.

This investigation was undertaken to determine the natural content of copper, zinc, iron, lead and arsenic in unsprayed apples, and the quantities of these metals which are likely to be found in juice and cider, arising from the natural metallic content of the fruit and from unavoidable, although slight, contamination from processing operations.

Copper, zinc and iron are normally present in the apple. McCance and Widdowson (4) have reported the copper and iron content of English eating apples as 0·6–0·7 p.p.m. and 2·3–2·9 p.p.m. respectively, of Empire eating apples as 1·1–1·4 and 2·2–2·9 p.p.m. respectively, and of raw English cooking apples as 0·9 and 2·9 p.p.m. respectively. The figures for English apples are slightly higher than those published in this investigation. Zinc has been reported as 0·26 p.p.m. in the peel and 0·33 p.p.m. in the flesh of apples by Sylvester and Hughes (5). In the apple varieties studied in this investigation zinc was found to vary from 0·21–0·76 p.p.m. Lead appears to be present in the apples studied in small but variable amounts up to 0·16 p.p.m., and arsenic in amounts less than 0·04 p.p.m.

The amounts of copper found in the cider apple juices were invariably higher than those in the fruit. This may be attributed to an uptake of copper during pressing, possibly by solution of the metal from the copper rivets in the press racks. The amounts of copper taken up by the juices were in general greatest in the more acid juices. The juices showed no corresponding increase in zinc content, nor did the amounts present bear any relation to the pH of the juices.

The amounts of copper and zinc in the ciders were, with a few exceptions, lower than those in the corresponding juices, suggesting that there had been some removal of these elements in the lees. The amounts of iron and of lead showed no consistent trend during processing and the slight increases found in individual ciders may have arisen from chance contamination.

At the time of writing, the limits for copper, lead and arsenic which have been recommended by the Metallic Contamination Sub-Committee of the Food Standards Committee are as follows: apples, arsenic 1·0 p.p.m. (as As); cider, copper 7 p.p.m.; lead provisionally 1·0 p.p.m., to be reduced within twelve months to 0·5 p.p.m., and arsenic 0·2 p.p.m. (as As). It will thus be seen that the ciders made from unsprayed apples in these tests have contained quantities of these elements well below the limits quoted above.

Summary.

(1) The natural content of copper, zinc, iron, lead and arsenic in seventeen varieties of apple has been investigated.

(2) The amounts of these elements present in the freshly-expressed juices and ciders prepared from the fruit have also been determined.

(3) In the fruit, the copper content varied from 0·27–0·59 p.p.m., the zinc content from 0·21–0·76 p.p.m., and the iron content from 1·0–2·5 p.p.m. Lead was present in small but variable amounts from 0·02–0·16 p.p.m., and arsenic was present in amounts less than 0·04 p.p.m.

(4) In the freshly-expressed juices, copper was slightly higher than in the fruit, suggestive of contamination during pressing ; the amounts of the other elements were not consistently affected.

(5) In the ciders the amounts of copper and zinc were usually lower than in the corresponding juices. The amounts of the other elements were not consistently affected.

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STUDIES ON CIDER SICKNESS.
II. THE EFFECTS OF HEAT TREATMENT OF CIDERS ON THE INCIDENCE OF SICKNESS.

By B. T. P. BARKER.

Introduction.

In this first paper of this Series (1) it was shown that (a) after each of twenty ciders used in the investigation concerned had been heated to 100° C. for five minutes and then infected with the sickness bacillus after cooling, in no case did any outbreak of the disorder result, (b) when the ciders were similarly treated after neutralisation by the addition of $CaCo_3$ before heating outbreaks occurred in all but three, and (c) when neutralisation without any heat treatment was applied every cider responded positively with an outbreak after infection.

In another investigation on the possible role of enzyme action similar treatments at temperatures ranging from 55° C. to 100° C. were tested. Sickness developed after heating to 80° C. and upwards, the outbreaks being most vigorous at 100° C. : at the lower temperatures no sickness appeared. The unheated ciders gave active outbreaks, although in some instances these were partly obscured by the development of yeasts and acetic bacteria. Further trials with other ciders of similar age gave corresponding results.

The above tests were made during 1948 and the early part of the summer of 1949, and the initial results given in the 1948 Annual Report (2). In the late summer and early autumn of 1949, when the ciders had matured further in bottle, the tests were repeated. The results then varied somewhat with individual ciders and also with the age of the liquor, but the general course was similar for each individual cider. It indicated that the effects of heating within the temperature range 55-75° C. gradually changed with increasing age of the liquor. The first signs of change were shown by the behaviour of the cells of the organism used as the inoculum. Instead of the individual cells remaining passive and normally dying in a short period, as happened in the previous trials, they showed slight signs of slow multiplication combined with lazy motility, traces of turbidity in the liquor and, sometimes, fermentation resulting. Rather later, multiplication and motility became more pronounced and turbidity and fermentation more definite, although still slower in onset than in the corresponding ciders of the same age unheated or heated to temperatures of 80–100° C. With still older samples active growth and fermentation occurred in all samples whatever the temperature of the heat treatment, although usually the fermentations of those of the 55–75° C. range were slightly less active and slower in onset than in those of other temperatures. Thus the nature of the response to heat treatment is associated in some way with the age of the cider.

These early observations were made on ciders which already had passed through the active fermentation stage and had been filtered and bottled, according to the usual practice at the Station, after the specific gravities had fallen upwards of 20 points. For the important period of their life prior to that stage information was lacking. Therefore, when freshly expressed juices in the autumn of 1950 became available, the work was resumed and the behaviour of individual juices throughout their fermentation life examined. The results of these later trials, carried out under modified conditions in an attempt to accelerate the age changes, are described below.

Heat Treatment Tests, 1950-51.

Three ciders made at the Station from the 1950 crop of three varieties of apples of widely different composition and vintage quality—Ashton Brown Jersey, Kingston Black and Bramley's Seedling—were selected for examination. The first-named variety is a bittersweet, normally giving juices of low acid and medium tannin contents with slow to medium rates of fermentation. Kingston Black belongs to the bittersharp class; its juices generally have acid and tannin contents of about 0·5 and 0·25 per cent. respectively, and rates of fermentation on the slow side. Bramley's Seedling, although not a cider apple proper, is widely used for cider making; its juice normally is excessively acid and extremely low in tannin, and its fermentation rate mostly very rapid.

The analyses of the three 1950 juices were as follows :—

	Specific Gravity.	*Acid* %.	*Tannin* %.	*Fermentation Rate.*
Ashton Brown Jersey ..	1046	0·13	0·17	1·3
Kingston Black ..	1062	0·47	0·17	1·0
Bramley's Seedling ..	1045	0·82	0·07	2·5

To accelerate the fermentation cycle a somewhat different procedure

from that adopted for the earlier work was used. The juices were brought direct from the press into the laboratory and kept under observation there in one-gallon glass jars : from these samples for the tests were drawn as required from time to time. Under the warmer conditions there fermentation of the juices proceeded much more rapidly than at the very low temperature of the ciderhouse. The gravity of the Bramley's Seedling juice, for example, dropped to 1003 in a fortnight ; fermentation then was practically at an end and natural clarification of the liquid had begun. The gravities of the Ashton Brown Jersey and the Kingston Black samples at the same date were respectively 1025 and 1036 and fermentation was still in progress.

Another difference in procedure was that fermentation was allowed to proceed unchecked until it reached its natural end. Throughout the duration of the trials the liquor was left in the original glass vessels undisturbed—apart from the withdrawal by pipette of the samples needed for the heat treatment tests. Thus the whole of the yeast crop produced during fermentation and all suspended solid matter, such as fragments of fruit tissue present in the original juice and pectin derivatives thrown out of solution, remained in contact with the liquor and contributed to it any soluble products resulting from chemical change, e.g. products of yeast antolysis. This procedure was adopted so that such of the products as might be utilisable in the metabolism of the sickness organism could make their contribution to its growth.

Three Series of heat treatments of these ciders were arranged at the following stages of development : I. Unfermented juice, II. Declining primary fermentation, III. Post-fermentation.

In each case their behaviour was examined at five temperatures—(a) ordinary temperature, (b) 60° C., (c) 70° C., (d) 80° C. and (e) 100° C.—after prior neutralisation by the addition of excess calcium carbonate. In addition, certain variants of the heat treatments were included in the trials ; these will be considered separately owing to the special features involved.

The tests were made in triplicate on 10 c.c. of the respective ciders contained in test tubes. In all cases the inoculum was taken from a 24-hour-old culture of the organism grown at 25° C. in a glucose-yeast extract solution. The inoculum consisted of one drop of the culture in each tube. After infection the ciders were kept at 25° C. throughout the duration of the tests.

Table I contains the summarised results for each of the three Series of the ciders under examination, set out in a form to facilitate comparison.

In Table I in addition to the times of first visual signs and cessation of fermentation of the respective samples which can be given with precision, an attempt to indicate the daily degree of vigour of the fermentation throughout its course has been made, since the individual cultures clearly showed material differences in that respect. It was found possible to distinguish approximately by the rate of the gas evolution the following eight distinct degrees of vigour :—1, very slow ; 2, slow ; 3, moderate ; 4, fair ; 5, active ; 6, vigorous ; 7, very vigorous ; 8, violent. Such classification can only be approximate, but it served its purpose for an investigation of the present order where exact quantitative results were not requisite at this stage of the work and direct comparison of the fermentation vigour could be assessed fairly closely by visual observation.

The outstanding features of the behaviour of the varieties under trial at

TABLE I.

Comparison of Vigour of Fermentation.

Days after Infection	Unheated			60° C.			70° C.			80° C.			100° C.		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Series I.															
1st	–	-	4	–	–	–	–	–	–	–	–	–	–	–	–
2nd	7	7	7	6	6	–	6	6	4	6	6	4	6	6	6
3rd	7	7	7	6	6	6	6	6	6	6	6	6	6	6	6
4th	7	7	7	6	6	6	6	6	6	6	7	6	6	7	7
5th	–	2	2	–	2	2	–	2	2	–	2	2	–	2	2
6th	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Series II.															
1st	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2nd	tr	3	6	3	3	–	–	3	–	4	6	tr	5	6	5
3rd	5	7	7	3	5	1	3	5	1	4	7	4	3	7	8
4th	5	4	6	1	5	5	1	5	4	–	7	6	–	7	8
5th	–	–	3	–	6	–	–	5	3	–	7	4	–	7	7
6th	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Series III.															
1st	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2nd	–	4	–	tr	4	–	–	4	tr	–	tr	–	4	4	–
3rd	4	5	2	tr	3	–	2	2	–	2	1	–	6	7	3
4th	5	7	3	3	6	tr	4	5	tr	3	5	tr	6	7	5
5th	5	7	6	6	6	2	6	5	1	6	5	–	4	7	6
6th	3	3	5	3	4	3	4	3	2	4	3	2	2	5	5
7th	–	2	3	1	2	4	2	2	3	2	2	3	–	2	4
8th	–	–	1	–	1	5	–	1	4	–	1	4	–	–	2
9th	–	–	–	–	–	–	–	–	–	–	–	5	–	–	1
10th	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Notes : A = Bramley's Seedling.
B = Kingston Black.
C = Ashton Brown Jersey.
tr = Signs of incipient fermentation.
1 – 8 = Grades of fermentation vigour in ascending order.
– = No fermentation.

the three selected stages in the life cycle of their ciders can be summarised as follows :—

Series I.

As a whole the results were remarkably close, both in the similarity of the general behaviour of the three varieties and the uniformity of the temperature responses.

Onset of Fermentation. Ashton Brown Jersey was one day in advance of the others with its unheated samples, but definitely slower than either for the temperature ranges from 60–80° C.

Intensity of Fermentation. Vigour was highest and equal in all cases in the unheated samples, in the 80° C. and 100° C. Kingston Black and the 100° C. Ashton Brown Jersey. In the remainder it was only one grade lower. A general feature throughout was the rapid attainment of the peak period.

Duration of Fermentation. All the Bramley's Seedling juices completed their fermentation in three days, being practically at their peak intensity throughout : the Kingston Black differed from them only in extending into a fourth day during subsidence. The Ashton Brown Jersey juices were rather

less regular, showing a briefer peak period in the 60–80° C. range but finishing, like the Kingston Black, on the 5th day after infection.

Series II.

The variations in the results from the varietal point of view were considerably greater than those of Series I and the treatment effects were more conspicuous.

Onset of Fermentation. No gas evolution in any of the tubes was visible until the second day : for the 70° C. Bramley's Seedling and the 60° C. and 70° C. Ashton Brown Jersey none appeared until the third day.

Intensity of Fermentation. In general it was lower and less prolonged than in Series I. Kingston Black at 80° C. and 100° C. and Ashton Brown Jersey at 100° C. stood out as marked exceptions, especially the latter which for two days reached a pitch of intensity higher than any other in the whole Series. Bramley's Seedling was notably weak, especially in the 60–80° C. range.

Duration of Fermentation. Three of the Bramley's Seedling set only showed visible fermentation for two days and the remaining two barely extended into a third day. The Kingston Black group were regular, the unheated samples covering three days and the remainder four. The Ashton Brown Jersey extended over four days in the unheated and 80° C. and 100° C. instances, but were reduced to two days and three days respectively in the 60° C. and 70° C. groups.

Series III.

The features of this Series were a fairly general delay in the first appearance of gas evolution and, in particular, a marked prolongation of the fermentation period, the peak duration mostly being short-lived.

Onset of Fermentation. Only in the cases of Kingston Black unheated and 100° C. and Bramley's Seedling 100° C. was any material gas evolution produced before the third day : for the three Ashton Brown Jersey, 60° C., 70° C. and 80° C., it was as late as the 5th day before gas evolution became obvious.

Intensity of Fermentation. The Kingston Black generally were definitely the strongest and the effects at 100° C. were more intense than in Series I and II. Bramley's Seedling and Ashton Brown Jersey showed some gain on Series II, but fell somewhat below the Series I standard.

Duration of Fermentation. In all cases the fermentation was more prolonged than in either of the previous stages and extended into the 9th day after infection in the Ashton Brown Jersey at 100° C.

Series IV.

This Series was carried out in conjunction with the first Series already described. Its purpose was to ascertain the effects of (a) leaving the unfermented juice unneutralised at both the unheated and the 100° C. levels. and (b) neutralisation after heating to 100° C., instead of before heating as in Series I. The juice used was Ashton Brown Jersey, and the tests were run concurrently with those of Series I. Thus direct comparisons were possible with the " unheated neutralised " and the " 100° C. neutralised before heating " juices of Series I which are included in the Table for that reason, The results are summarised in Table II.

TABLE II.

Ashton Brown Jersey Cider.—Unfermented Juice.

Days after infection	Unheated		Heated to 100° C.		
	Not neutralised	Neutralised	Not neutralised	Neutralised before heating	Neutralised after heating
1st	–	4	–	–	–
2nd	–	7	–	6	–
3rd	7	7	–	6	–
4th	7	7	2	7	2
5th	2	2	4	2	4
6th	–	–	4	–	4
7th	–	–	2	–	2
8th	–	–	–	–	–

In the unheated juices there was a difference of two days in the appearance of active fermentation, the earlier being the neutralised juice : in both all signs of fermentation had disappeared by the 6th day after infection. Yeast action undoubtedly was partly responsible for the fermentation, but the more rapid onset in the neutralised juice suggests that the sickness bacteria at that more favourable pH level may have contributed largely, if not entirely, to that result.

In the three cases where heating to 100° C. was involved, there was similarly a gain of two days in the appearance of fermentation and a marked increase in fermentation intensity in the juice neutralised prior to heating as compared with the other two : subsidence in fermentation became obvious in each case four days after the onset of fermentation. From this result it would appear that neutralisation before heating has altered the character of the juice not only as regards its pH during the heat treatment but in some other respect, making it thereby a more favourable nutrient medium for the bacillus than the other two 100° C. juices. The difference in behaviour of these was so slight as to be negligible, thus showing that the lower pH of the unneutralised juice was nevertheless sufficiently high after treating not to impair the growth of the organism.

Series V.

The object in this case was to see if the changes in sickness susceptibility resulting from the heat treatments of the neutralised ciders in Series III were associated with the time of neutralisation. The ciders tested were the same as in Series I and III, and the liquids at the time of testing were approximately at the same stage as those used in Series III. Four sets of treatments were applied and each tested at two different temperatures, 70° C. and 100° C. The respective treatments were :—

(A) heating without neutralisation.

(B) neutralisation with $CaCo_3$ before heating.

(C) heating before neutralisation and subsequent neutralisation with $CaCo_3$ after cooling and before infection.

(D) heating before neutralisation and then subsequent neutralisation while still hot, with further heating at the same temperature for a period of five minutes before cooling and infection.

The results are summarised in Table III and the outstanding features shown in the tabulated results are as follows :—

Treatment (A) No sickness developed in any of the unneutralised ciders heated to 70° C. nor in the Bramley's Seedling 100° C. The Kingston Black in the latter case gave only a moderate fermentation and the Ashton Brown Jersey even less : in both instances onset was delayed until the 4th day and the duration was prolonged, especially in the Ashton Brown Jersey.

Treatment (B) The ciders neutralised before heating showed no marked differences between the results for either temperature treatment except in the case of the Ashton Brown Jersey 70° C. The others reached the fermentation peak in 3–4 days after an early onset, and the duration was only moderately prolonged, except in Ashton Brown Jersey 100° C., and the intensity ranged from " active " to " vigorous." The onset in the Ashton Brown Jersey 70° C. was delayed until the 7th day and fermentation had ended three days later : its vigour was less than that of the others.

Treatment (C) For these ciders, neutralised after heating, there were greater divergences than in those of the (B) group. At 70° C. fermentation was slower in reaching its peak and was more prolonged for the Kingston Black and Ashton Brown Jersey ; intensity in the respective cases was as under (B) : Ashton Brown Jersey again showed delayed onset until the 7th day. At 100° C. Bramley's Seedling and Kingston Black gave results as under (B), but Ashton Brown Jersey differed in its behaviour by a tardy onset and delayed peak similar to its (B) and (C) performance at 70° C.

Treatment (D) Neutralising after heating and then reheating resulted in a very close similarity with the (B) behaviour of Bramley's Seedling and Kingston Black for both the 70° C. and the 100° C. samples as regards onset, peak and duration periods, but fermentation intensity in general was appreciably stronger. The latter feature also applied to the two Ashton Brown Jersey samples, but whereas the behaviour of the 70° C. sample closely matched that under treatments (B) and (C) the 100° C. cider followed the pattern of (B) and not of (C).

Reviewing the results as a whole, treatments (B), (C) and (D) showed very little difference in their effects on the Bramley's Seedling and Kingston Black respectively, either at 70° C. or 100° C., or on the Ashton Brown Jersey at 70° C. The Ashton Brown Jersey at 100° C. showed considerable uniformity under treatments (B) and (D), but a very significant difference under (C). In general treatment (D) was associated with an appreciable increase in intensity of fermentation.

Bramley's Seedling and Kingston Black behaved remarkably alike except in the unneutralised treatment (A) at 100° C. : the much greater degree of acidity of the Bramley's Seedling doubtless accounted for the difference, in view of the organism's intolerance of a low pH. Both varieties belong to the

TABLE III.

EFFECTS OF HEAT TREATMENT BEFORE OR AFTER NEUTRALISATION.—POST-FERMENTATION CIDER.

Cider	Fermentation Period in Days																Order of Fermentation Vigour
	A				B				C				D				
	Not Neutralised				Neutralised												
					Before Heating				After Heating								
									Not Reheated				Reheated				
	Start	Peak	End	Degree of Vigour	Start	Peak	End	Degree of Vigour	Start	Peak	End	Degree of Vigour	Start	Peak	End	Degree of Vigour	
Bramley's Seedling																	
70° C.	—	—	—	—	3	3	8	Vigorous	3	6	8	Active	3	5	8	Vigorous	B & D = 1st, C 2nd
100° C.	—	—	—	—	2	3	7	Vigorous	2	4	7	Vigorous	2	4	6	Very Vigorous	D 1st, B 2nd, C 3rd
Kingston Black																	
70° C.	—	—	—	—	2	3	7	Vigorous	3	5	10	Vigorous	3	4	8	Very Vigorous	D 1st, B 2 nd C 3rd
100° C.	4	5	9	Moderate	2	4	6	Vigorous	2	4	6	Vigorous	2	4	6	Very Vigorous	D 1st, B & C 2nd, A 3rd
Ashton Brown Jersey																	
70° C.	—	—	—	—	7	8	10	Fair	7	9	11	Fair	7	9	10	Active	D 1st, B & C 2nd
100° C.	4	5	12	Slow	2	4	9	Active	6	6	11	Vigorous	2	3	10	Vigorous	D 1st, B 2nd, C 3rd, A 4th

" sharp " class, whereas the Ashton Brown Jersey is a bittersweet of low acidity. There is considerable support for the conclusion that the " acidity " factor has played an important and, possibly, predominant part in the results ; for in another paper in the present Report (3) corresponding treatments with another bittersweet variety, Yarlington Mill, have given results practically identical with those of the Ashton Brown Jersey in this investigation.

Summary and Conclusions.

The results of the above work, considered in conjunction with those on heat treatment previously recorded, make it possible now to form some idea of their significance in relation to the series of changes taking place in cider during the course of its life. These changes unquestionably affect its degree of susceptibility to the sickness disorder. It seems that during the normal natural fermentation life of a cider the liquor as a medium of growth of the sickness bacillus—after its pH has been adjusted to a favourable level for the organism—passes through a sequence of susceptibility alterations which conform more or less closely to a definite pattern. Individual ciders differ in the degree of change ; that is to be expected from the wide ranges of difference in their initial chemical composition.

In general terms this sequence takes the following course. The freshly expressed juice, after suitable pH adjustment, is a good medium for the growth of the organism. As the primary fermentation passes to and beyond its peak of intensity, the liquor becomes less favourable in certain respects, but with advancing age gradually regains to some extent its original suitability, although the rate of growth of the bacillus is lessened appreciably. The special effects of the individual heat treatments on the liquor at those three stages are discussed separately later, but in general they fall in line with the above observations.

In the early life of a cider the growth of yeasts obscures the behaviour of the sickness bacteria—which are probably generally present in the freshly expressed juice from the start—and normally the latter do not come into prominence until yeast action has diminished naturally—or artificially by centrifuging or filtration. Practical experience indicates that so long as some degree of yeast activity continues ciders can resist sickness attack ; but when it slackens there is danger. It is interesting to note that this accords with the observations of the previous paragraph.

The sequence of changes in the unheated neutralised ciders as nutritive media for the growth of the bacillus appears to be more definite and intensified in the corresponding ciders subjected to heat treatment, particularly at temperatures between 60° C. and 80° C. From 80° C. to 100° C. the vigour of growth runs more nearly parallel to that of the unheated ciders at each of the stages examined. The results obtained in the earlier stages of the work, when a group of twenty bottled ciders filtered before the primary yeast fermentation had been completed were used for the tests, were more pronounced than those yielded by the Bramley's Seedling, Kingston Black and Ashton Brown Jersey ciders, which were allowed to ferment to completion and remain unfiltered and standing on the lees. In the former case, for example, the ciders in middle life after being heated to 60° C.–70° C. gave no growth of the bacillus.

The nature of the changes in the ciders during their life cycle which affect the growth of the organism to produce the results recorded is still obscure in many respects. As regards the carbohydrate metabolism of the bacillus there is no problem, since an adequate supply of glucose has been available at all stages. For its nitrogenous metabolism considerable variations in the nitrogen supply have taken place. At the " unfermented juice " stage there has been available the initial nitrogen of the juice, about 80 per cent. being quickly assimilated by the yeasts within a few days after juice expression and the remainder showing little change in quantity during later life, as shown originally by Dickson (4) and confirmed by Challinor and Burroughs (5). The vigorous growth of the sickness organism in unfermented juice of suitable pH suggests that it also can utilise without difficulty the " readily assimilable " portion just mentioned. The position with regard to the residual nitrogen—1·5–2 mg. 100 ml. in amount—is less clear : considerable growth of the bacillus occurs in older neutralised liquors, both when unheated and after heating to 80° C.-100° C., although some loss of vigour as compared with the unfermented juices has been noted at those temperatures and has been more pronounced in the samples treated to 60° C.–75° C. Further, in the case of the bottled ciders referred to above it was found that both when heated to 100° C. without neutralisation (1) and to 60° C.–75° C. after neutralisation no growth of the bacillus occurred. (2). (In the former, growth in several cases occurred in unheated samples without neutralisation). Even if in the unheated samples, with or without neutralisation, the residual nitrogen had contributed in some degree to the growth of the organism, the results for the heated samples show definitely that some change brought about by heat has taken place. This suggests that a possible contribution to the nitrogen contents at this stage in the life of the cider, in the form of products of yeast autolysis, is negligible, because the organism can grow very freely when small quantities of yeast extract with or without heating are supplied as the sole source of nitrogen. On the other hand it seems probable that these autolysates may take a significant part in the increased growth results recorded for the tests on post-fermentation ciders which had remained for a considerable period on the moribund yeast crop in the lees. Nevertheless, whether directly or indirectly, the source of the nitrogen required for the growth of the organism at any period in the life of the cider must be the initial supply in the freshly expressed juice : also some part of it or its derivatives must undergo changes under heat treatment at 60° C.–75° C. adversely affecting its assimilable availability for the bacterium, unless some other nutrient deficiency—or a toxic action, for which at present there is no direct evidence—is responsible.

A very important aspect affecting the development of the bacterial crop is that of accessory growth factors. The work on this subject is not yet sufficiently advanced for publication, but results recently obtained indicate that one of those factors in particular, viz., pantothenic acid, must be taken into account. Sickness bacteria can grow to a limited extent in aqueous solutions containing only glucose and inorganic salts supplying the basic elements N, P, K, Mg and Ca plus any traces of micronutrient elements present as impurities in those ingredients. Consequently they must be capable of synthesizing the essential growth factors to some extent, even if only at sub-optimal amounts. The addition of Ca pantothenate to such solutions produces an immediate striking increase in the growth and fermentation

activity of the organism. The only information on growth factors in ciders hitherto published (6) relates to the content of biotin, nicotinic acid, riboflavin and pantothenic acid in ciders over one year old : it thus has little bearing on the results recorded in this paper beyond showing that probably the two former were present and the two latter might have been.

Knowledge of the other individual nitrogenous constituents of apple juice and cider is incomplete. Asparagin is the predominating substance and accounts for about 70 per cent. of the total nitrogen : the remaining N is mainly in the form of amino acids (7). These are probably sufficiently thermostable for them not to be accountable for the growth failures of the bacterium which occurred at the 60° C.–75° C. range.

The requirements of the bacterium for phosphorus are not high and even matured ciders contain sufficient of that element to support growth. The other essential macro-nutrients, potassium and magnesium particularly, are also present in adequate amounts. The micro-nutrient elements essential for growth are at present under investigation in relation to the organism's requirements, but so far there has been no indication that any of them are deficient.

There may be some significance in the differing results given in some of the tests by the three varieties under trial. As a whole, Bramley's Seedling and Kingston Black have been fairly in accord, while Ashton Brown Jersey has been more at variance ; on the other hand Yarlington Mill, in another set of tests, has shown almost identical behaviour with Ashton Brown Jersey. Those two varieties are characterised by the relatively low acidity of their ciders. Thus the acid content of a variety is a factor which may have some indirect bearing on the results, apart from its direct pH effects on the organism itself.

While the " tannin " contents of the Bramley's Seedling ciders were far lower than that of the other three varieties, there is little evidence from the results of the trials to suggest that this difference had any material bearing on them.

From this survey of the various nutrient aspects of the ciders under trial, although the results for the " unheated " treatments and most of those in the " 100° C. heated " group are fairly in line with the known nitrogen cycle in the respective liquors as they pass through the phases of their fermentation life, no obvious feature has been forthcoming to account for the " 60° C.–70° C. heated " group results during the middle period. This makes it difficult to correlate the results absolutely with that cycle and points to other " age " changes during the transition period from the unfermented juice to the finished and matured product, the nature of which is obscure at present. Such changes were more marked in their effects on the degree of sickness susceptibility of ciders filtered before the end of the natural primary fermentation, as compared with those left unfiltered and standing on the lees.

Also the resistance to sickness shown by ciders of corresponding age after being heated unneutralised to 100° C. may be of significance in this connection, especially when considered in conjunction with the enhanced degree of sickness intensity when ciders thus treated were subsequently neutralised and then reheated to 100° C. It is certainly suggestive that in the latter case the initial heating was given to the liquor in its acid condition, which evidently is more favourable for the change than when neutralised.

The work on the Mo content of Yarlington Mill referred to above possibly

sheds some light on this point. It shows (a) a rise in the Mo content of freshly expressed juice after immediate sterilisation and (b) a drop in Mo content after sterilisation and subsequent storage for three months. Thus there is definite evidence of both Mo precipitation on standing and its solution from its precipitated state on heating the unneutralised liquor. Mo, as a micro-nutrient, acts as a growth stimulant to the sickness organism. What has been demonstrated for this element may apply to other essential or stimulant micro-nutrients present in apple juice and affect materially the susceptibility of the liquor to sickness at different stages of its life history.

From this discussion of these results, taken in conjunction with previous knowledge, in their bearing on the problem of the liability of any individual cider to an outbreak of the disorder, the following conclusions seem justified :—

(1) The degree of susceptibility varies with the age of the liquor.

(2) The chemical composition of a cider regarded as a nutrient complex for the sickness organism differs according to age, particularly in respect of its content of nitrogen, phosphorus, potassium and probably magnesium, molybdenum and possibly other stimulant micro-nutrients.

(3) The pH status changes with age. Normally only the low-acid ciders of the sweet and bittersweet classes are susceptible : but those of somewhat lower pH may be attacked, if the nutrient complex is unusually favourable for the growth of the organism, especially if the growth factor, pantothenic acid, is present in adequate amount. Absence of the latter greatly reduces the risk of outbreak.

(4) The most favourable period for an outbreak is after primary fermentation has ceased, especially if the cider is allowed to remain on the lees. In such cases, with high N juices little or no sugar remains in the fermented cider, which consequently escapes attack : with low N juices yeast action ceases before the whole of the sugar is destroyed and the chances of outbreak are considerable.

When ciders are not allowed to stand on the lees after primary fermentation is ended and are then, or earlier, subjected to clarification treatment to retain some natural sweetness, the risk of sickness is high for low-acid liquors. The sooner after clarification the ciders are bottled, the greater is the chance of avoiding an outbreak : storage in the wood appears to be favourable for its development.

With the present information now available it is possible to understand more clearly why certain ciders succumb to the disorder and others derived from juices of apparently similar chemical composition remain immune. Much further knowledge is required before complete control can be assured ; but in the meantime a lead as to suitable conditions of management is indicated.

A feature of more general interest disclosed by these investigations is the sequence of certain age changes in cider as it proceeds to maturity. What their significance may be in relation to the quality of the fully matured beverage has yet to be discovered. It seems likely, however, that equivalent changes occur during the maturing of wines and other fermented fruit beverages: in that case their further study assumes a considerably wider importance.

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STUDIES ON CIDER SICKNESS. III. THE EFFECTS OF TREE INJECTION WITH MOLYBDENUM ON THE COMPOSITION OF APPLE JUICE AND ITS SUSCEPTIBILITY TO CIDER SICKNESS.

By B. T. P. BARKER, D. J. D. NICHOLAS and W. PLANT.

Before the publication of the results of a study of molybdenum content of ciders in relation to the incidence of the disorder of cider sickness (1), little or no information on the amount of Mo in apple juices and ciders was available. Injections of apple trees with sodium molybdate were made in 1948 with the object of finding if the Mo content of the fruit was increased thereby. The present paper deals with (a) the results on that point shown by the crops from treated and untreated trees, and (b) the susceptibility of the respective juices to cider sickness. So far as the latter aspect is concerned, the results supplement those reported in the afore-mentioned paper, which showed that the Mo levels of the ciders then examined were sufficiently high to ensure that sickness was not retarded by a deficiency of that element. The present investigation has shown how the sickness organism was affected by the higher Mo levels in the juice from injected trees.

Two bittersweet varieties of cider apples—White Jersey and Yarlington Mill Jersey—were selected for the trials, their low-acid juices being susceptible to outbreaks of cider sickness. In both instances an untreated tree of similar age and general character was available adjacent to the Mo treated tree. Furthermore the trees were sufficiently young and vigorous to permit ready translocation of the sodium molybdate.

Four trees of each variety were reserved for the experiment, three being injected with sodium molybdate and the other being left untreated. Their approximate ages were 15-20 years. The injections were made on 28/3/1949, the procedure being as follows. Branches 5″–6″ in diameter were used and 2-3 holes $1\frac{1}{2}$″ deep, were made, using a 7/16″ bit. The holes in each branch received in all 1 gram sodium molybdate and were subsequently stopped with corks. Two branches, placed opposite and as far away as possible from the injected branches, were chosen as untreated controls on the injected trees.

In the autumn of 1949 the crops of both varieties from the treated and untreated branches of the injected trees and from the control trees were sufficient for a preliminary examination of the expressed juice of the fruit. Enough fruit for a more detailed examination was available from each of the Yarlington Mill trees in 1950 : the White Jersey trees bore no fruit that year.

Section I. Molybdenum Content of Juices.

All juices of the 1949 crops showed slow rates of primary yeast fermentation at laboratory temperature. Those from the control fruit of both varieties were exceptionally slow and signs of natural outbreaks of cider sickness were noted in the Yarlington Mill juices. These features rendered them unsuitable for the programme of experimental work on sickness susceptibility which had been planned : accordingly the only details considered here are the specific gravities of the freshly expressed juices, their rate of fermentation and their molybdenum contents.

The 1950 juices, although slow-fermenting, showed appreciable differences in rate of primary fermentation and proved more suitable for comparative tests of sickness susceptibility.

The 1949 Juices.

White Jersey. Three juices of this variety were obtained, one being from fruit grown on the untreated tree, another from the fruit of the injected branches of the treated tree and the third from the crops of the uninjected branches of the same tree. The data concerning the respective juices are given in Table I. (In the later pages the symbols U, T.I and T refer respectively to the untreated tree, the treated tree—branches injected—and the treated tree—branches uninjected.)

Table I shows that the Mo contents of U and T were reduced to some extent by sterilisation of the juice, especially after a three months' interval following treatment. The possible significance of the changes is considered later.

No evidence that Mo affects juice gravity or fermentation rate is apparent in these cases ; nevertheless rates of the two juices, T and T.I, are similar in spite of a considerable difference in specific gravity. The contrast between those and that of the U juice is striking. The latter was so abnormally slow that the juice might be described as almost non-fermentable.

Yarlington Mill. As with the White Jersey, three juices from corresponding sources were examined. The data relating to them are given below in Table II.

The specific gravities of the freshly expressed juices in this case differed from those of White Jersey in that U was lower than the T and T.I. Further, instead of the considerable disparity between the T and T.I samples shown by the corresponding White Jersey juices, they were identical in this instance.

The Mo content of the juices, U and T, were alike, but that of T.I was over five times as great.

Thus for this variety, also, there was no correlation between specific gravity and Mo content.

The comparative rates of fermentation are similar to those of the White Jersey, the only difference being a greater fall of gravity for each of the

TABLE I. White Jersey Juices—1949.

Source of Juice	Specific Gravity of Freshly Expressed Juice 2/11/1949	Specific Gravity of Fermented Juice 8/12/1949	Rate of Fermentation. Average Daily Fall in Gravity at Laboratory Temperature	Molybdenum Content of Juice μg/ml.		
				Determined immediately after Expression		Determined after Sterilised Juice had been kept for 3 Months
				Unsterilised	Sterilised	
Control Tree	1,053	1,049	0·11	0·01	0·005	0·002
Injected Tree : Untreated Branches ..	1,046	1,026	0·55	0·02	0·01	0·003
Injected Tree : Treated Branches ..	1,054	1,033	0·58	0·05	0·05	0·05

TABLE II. Yarlington Mill Juices, 1949.

Sources of Juices	Specific Gravity of Freshly Expressed Juice 5/10/1949	Specific Gravity of Fermented Juice 7/12/1949	Rate of Fermentation Average Daily Fall in Gravity at Laboratory Temperature	Molybdenum Content of Freshly Expressed Juice μg/ml.	
				Unsterilised	Sterilised
Control Tree	1,048	1,020	0·44	0·01	0·005
Injected Tree Untreated Branches	1,060	1,006	0·86	0·01	0·005
Injected Tree Treated Branches	1,060	1,005	0·87	>0·05	>0·055

respective comparable juices. In other words the fermentation rate seems to be characteristic of the individual trees concerned and independent of their Mo status.

The 1950 Yarlington Mill Juices.

The corresponding data for these juices are given in Table III.

As with the 1949 juices, no obvious correlations between the Mo content and the other data appear. The exceptionally high Mo status of the T.I juice may have been associated with the relatively rapid fermentation of that juice. Thus, for the three sets of juices recorded in Tables I–III the highest Mo content has been usually associated with the highest fermentation rate. Nevertheless, as yet, too little is known of the quantitative aspects of the Mo requirements of the organisms concerned to justify any claim that Mo results in an increased rate in these instances.

Sickness bacteria were undoubtedly numerous during the concluding stages of the fermentation of the 1949 and 1950 juices, and for that reason the fermentation rates of the 1950 juices were calculated only to the 14th day after juice expression ; this period shows adequately the comparative rates during the most active yeast-fermentation stage. How far the bacteria may have affected the ultimate specific gravities it is impossible to say.

A further examination of the sterilised T.I juice of Yarlington Mill was made three months after the first examination. The contents of other micro-nutrient elements in the liquor in this instance were determined chemically at the same time. The results were as follows :—

Molybdenum ..	0·25	μg per ml.
Zinc	0·12	„ „ „
Copper ..	0·25	„ „ „
Iron	1·30	„ „ „
Magnesium ..	0·22	„ „ „
Lead	0·20	„ „ „

The Mo content had thus diminished by over one-half during the three months storage of the juice, a result considerably more marked than in the stored White Jersey 1949 juices of much lower initial Mo content.

Time and Extent of Mo Translocation.

The results for the Yarlington Mill 1949 juices showed that the Mo content of the T.I juice was relatively high, indicating that the element was being translocated to the fruit. The crops of the following year from those branches showed a further and very marked increase.

The T juices showed little, if any, increase in the Mo content in the first year, as compared with those from the adjacent untreated tree. In the second year, however, there was a marked increase, indicating that translocation of Mo had taken place. This increase did not approach in total amount that of the T.I juices.

The Mo level of the juices from the untreated trees remained practically unchanged during the two years under review.

The behaviour of the White Jersey trees in the first year were similar to those of the Yarlington Mill trees in that year. The increase in Mo content of the fruit from treated branches was of the same order for both varieties.

TABLE III.

YARLINGTON MILL JUICES, 1950.

Source of Juice	Specific Gravity of Freshly Expressed Juice	Specific Gravity of Fermented Juice (13th day)	Rate of Fermentation. Average Daily Fall in Sp.Gr. at Laboratory Temperature (to 13th day)	Molybdenum Content of Freshly Expressed Juice μg/ml.	
				Unsterilised	Sterilised
Control Tree	1,043	1,021	1·5	0·003	0·0025
Injected Tree : Untreated Branches	1,040	1,025	1·1	0·02	0·03
Injected Tree : Treated Branches	1,041	1,007	2·6	0·2	>0·5
				Molybdenum Content of Dried Pressed Pomace μg/g	
				3·68	

Section II. Sickness Susceptibility Trials.

Some of the juices described under Section I were further tested by heat treatment. The method is described elsewhere (2).

The juices examined were the three 1950 Yarlington Mill samples, which differed materially in Mo content. They were examined at two stages in their history :—(1) as " freshly expressed " juices and (2) as " fully-fermented " ciders. In the first case the heat treatment was limited to one temperature only, 100° C. ; in the second the tests were as comprehensive as the more detailed ones recorded in another paper in this Report (2).

The details and results of these tests were as follows :—

Test I.

Each of the freshly expressed juices used in this case was divided into three parts, the respective treatments of these being :—

(A) Direct infection of the unheated liquor with the sickness organism.

(B) Five minutes at 100° C. and infection after cooling.

(C) Neutralisation by addition of excess $CaCO_3$ prior to five minutes at 100° C. and infection after cooling.

The fermentation rates after treatment A were due partly to the sickness organism and partly to the live yeasts naturally present in the unheated juices.

The three graphs included in Fig. 1 show the character of the fermentation as regards the time of first visible appearance of activity, intensity and duration. They refer respectively to the treatments A, B and C.

First signs of fermentations in the juices in treatments A and B occurred between the second and third day : for the juice in treatment C it began about 24 hours sooner. The highest intensity of fermentation was given by the juice with the highest Mo content, T.I.

For the unheated juices (Fig. 1A) the other noteworthy feature is the closely allied character of the results for the two low-molybdenum juices—U and T—respectively.

For the two heated juices (Figs. 1B and 1C) the general resemblance of the graphs for the U and T.I juices is very close, differing only in a somewhat lower intensity of fermentation in the former (low Mo) juice. In Fig. 1C the T juice (low Mo), also shows a similar resemblance to T.I (high Mo), with fermentation intensity still somewhat lower ; it, however, under treatment B (heated and unneutralised), failed to support growth of the organism.

Test II.

The treatments given were more comprehensive than those in Test 1 and were carried out on the " fully fermented " ciders. The same general procedure was adopted, but in this instance the addition of 5% glucose was necessary to provide a supply of fermentable sugar for the organism, since none of the original sugar contents of the juices remained at the " fully fermented " stage.

The details of the respective treatments were as follows :—

(A) Direct infection of the unheated liquor.

(B) Infection after five minutes at 100° C. without neutralisation.

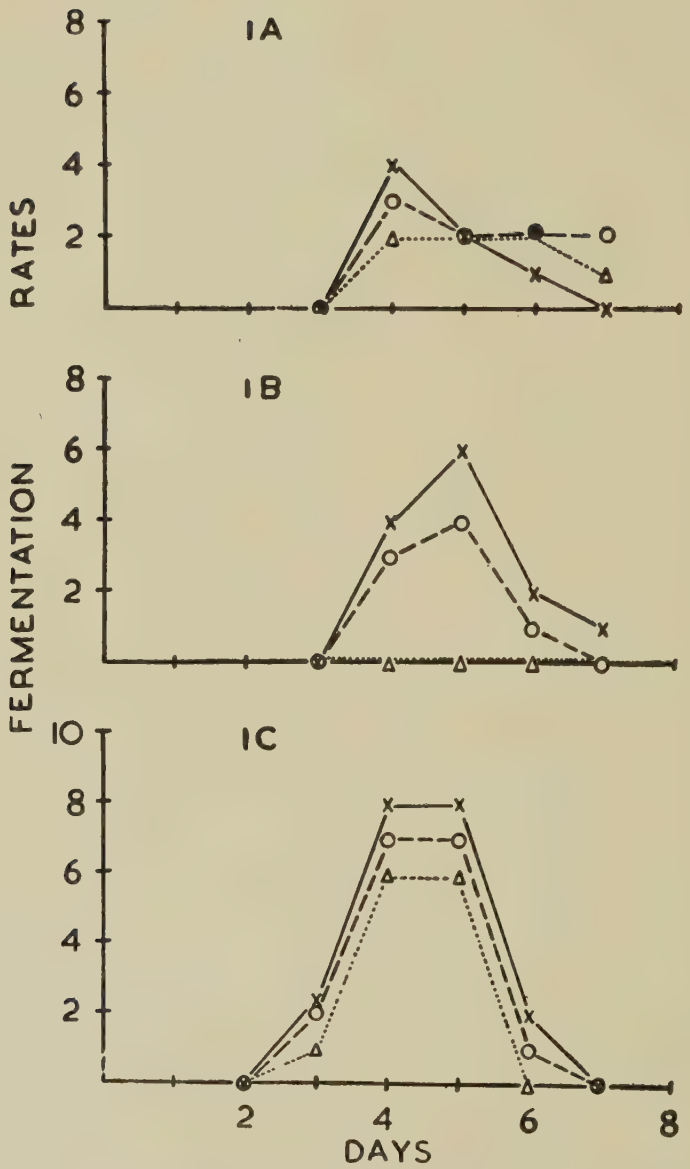

Fig. 1. Course of Fermentation of Freshly Expressed Yarlington Mill Juices.

(A) Juice unheated neutralised, and infected.
(B) Juice heated to 100° C. and infected after cooling.
(C) Juice neutralised, then heated to 100° C. and infected after cooling.

Key. Juice from untreated tree (U) – – –o– – –o– – –o– – –

Juice from untreated branches of the injected tree (T) Δ....Δ....Δ....Δ

Juice from treated branch of injected tree (TI) x——x——x——x——

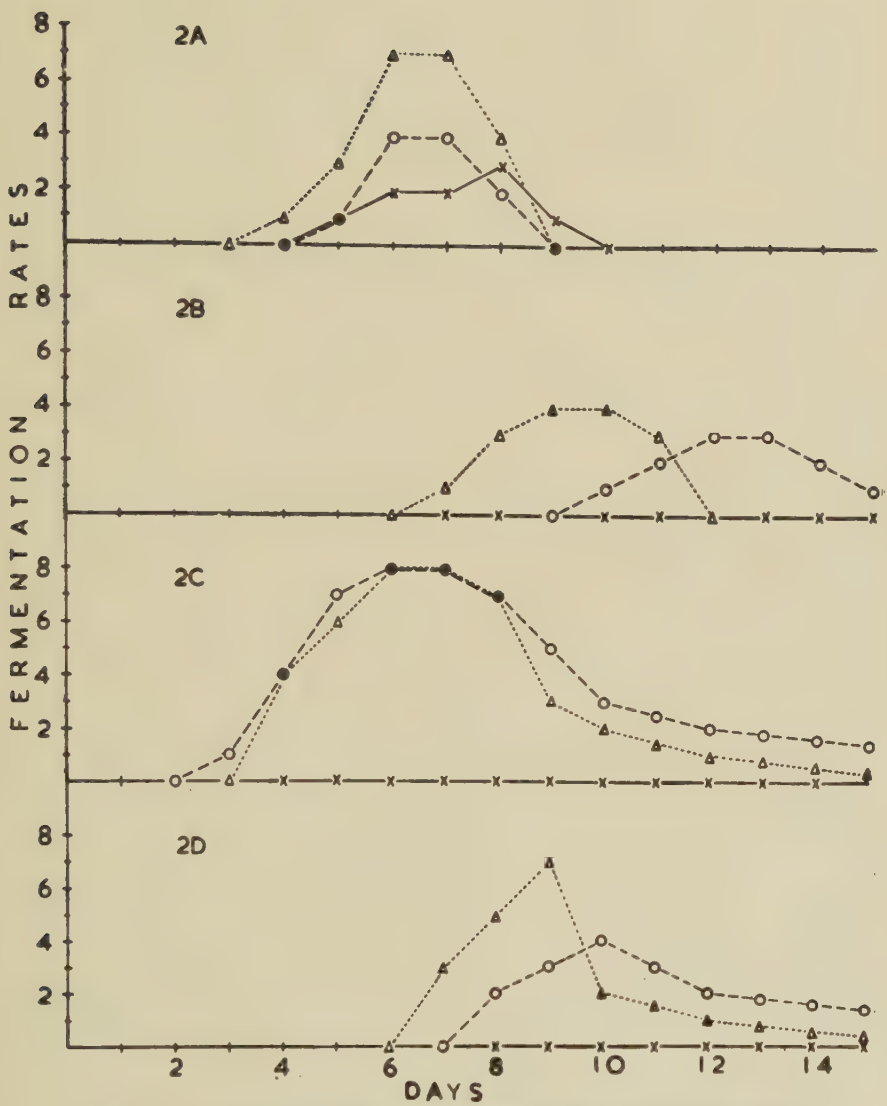

Fig. 2. Course of Fermentation of Fully Fermented Yarlington Mill Ciders.

(A) Cider unheated, neutralised and infected.
(B) Cider heated to 100° C. and infected after cooling.
(C) Cider neutralised, then heated to 100° C. and infected after cooling.
(D) Cider neutralised, then heated to 60° C. and infected after cooling.

Key. Cider from untreated tree (U) – – –o– – –o– – –o– – –.
Cider from untreated branches of the injected tree (T) Δ....Δ....Δ....Δ....Δ..
Cider from treated branch of injected tree (TI) ——x——x——x——.

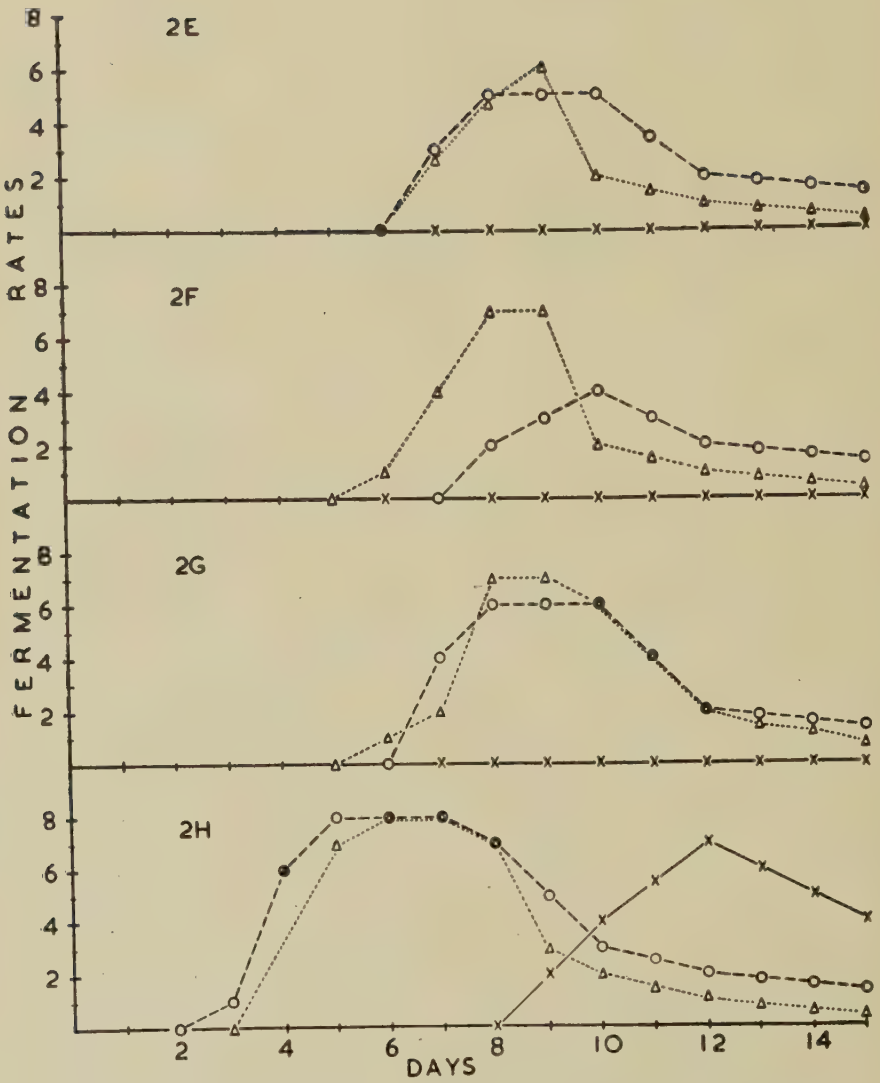

Fig. 3. Course of Fermentation of Fully Fermented Yarlington Mill Ciders.

(E) Cider neutralised, then heated to 70° C. and infected after cooling.
(F) Cider neutralised, then heated to 80° C. and infected after cooling.
(G) Cider heated to 100° C., then neutralised after cooling and infected.
(H) Cider heated to 100° C., then neutralised after cooling, reheated to 100° C., cooled and infected.

Key. Cider from untreated tree (U) – – –o– – –o– – –o– – –.
Cider from untreated branches of the injected tree (T) Δ. . . . Δ. . . . Δ. . . . Δ. . . . Δ
Cider from treated branch of injected tree (TI) ——x——x——x——.

(C) Infection after neutralisation with $CaCO_3$ and subsequent five minutes at 100° C.

(D) Infection after neutralisation with $CaCO_3$ and subsequent five minutes at 60° C.

(E) Infection after neutralisation with $CaCO_3$ and subsequent five minutes at 70° C.

(F) Infection after neutralisation with $CaCO_3$ and subsequent five minutes at 80° C.

(G) Infection after five minutes unneutralised at 100° C. and then subsequent neutralising after cooling.

(H) Infection after five minutes unneutralised at 100° C. and then subsequent neutralising after cooling and reheating for a further five minutes after neutralisation.

In all the heat treatments the liquor was allowed to cool to the ordinary laboratory temperature before infection. After infection all the ciders were kept at 25° C. and examined daily.

Three of the treatments —A, B and C—were identical with those applied in Test I and thus serve for direct comparison of the behaviour of the respective liquors in the " freshly expressed juice " and " fully fermented " stages. The corresponding graphs for each treatment show that there are radical differences in the behaviour of the liquors at the two stages of sampling. The outstanding feature is the performance of the " high Mo " T.I liquor.

The T.I Liquor. The fermentation predominance of this at the " freshly expressed " stage had disappeared completely in the " fully fermented " ciders : in fact under the treatments B and C no signs of fermentation had appeared by the 40th day after infection. Under the unheated treatment, A, some fermentation was noted, but it appeared a day later than in the other two juices and fell much short of them in intensity. How far the fermentation was due to the natural yeast flora of the unheated cider is difficult to estimate, although daily microscopic examination of the yeast and bacterial flora present has given some indication. Initially the multiplication of both yeasts and bacteria was slow. In the early stages the yeasts were predominant and the relatively few bacterial cells seen were unusually large, non-motile involution forms. By the 6th day the bacteria were beginning to constitute a major part of the micro-flora and a few motile diploid cell forms had appeared. The latter increased in number relatively quickly during the two following days, while the yeasts on the other hand had not shown any material increase. By that time the fermentation had reached its peak. Thus it would seem that while the earlier fermentation stages represented yeast effect mainly, the following more intense fermentation towards the peak period noted on the 7th and 8th days was largely the result of bacterial activity.

The graphs for the other five treatments, D-H, of the T.I liquor show no activity of the organism in any case except H, where fermentation did not appear until the 9th day ; but thence onwards for the next three days a relatively vigorous fermentation developed, which then slowly subsided but had not completely ended by the 17th day. At its peak it nearly reached the highest intensity attained in any of the tests with this liquor—Test I.C. " freshly expressed " juice heated to 100° C. after neutralisation.

The U and T Liquors. The other two liquors, U and T, on the other hand,

gave positive fermentations in both the A, B, C and the remaining D-H treatments.

The results in the former group, in comparison with those of the same treatments when applied to the " freshly expressed " juices, differed from the latter in the following respects :—

(i) A higher level of intensity and a more prolonged fermentation at that level were featured by both liquors at the " fully fermented " stage, but in each instance the first signs of fermentation were rather longer in making their appearance.

(ii) There was much greater retardation in the first appearance of fermentation under this treatment with the U liquor and the vigour level at the peak period was rather lower : on the other hand the duration of fermentation was far more prolonged.

For the T liquor a well-marked fermentation, very similar in duration and intensity to that just described for the U liquor, made its appearance three days sooner than the latter, whereas in the preceding test with the corresponding " freshly expressed " juice no visible fermentation occurred.

(iii) The fermentation graphs for both liquors were almost identical in general forms, the peak level being reached simultaneously and maintained for the same period of time and the total duration of fermentation being prolonged to an approximately similar length, the U cider starting a day ahead and finishing slightly later. In both cases the duration and peak level exceeded the corresponding features shown by the respective " freshly expressed " juice samples.

There was a more marked difference in fermentation behaviour of the two liquors for the D, E or F treatments which covered the temperature ranges of 60° C.–80° C. While in those cases the fermentation period did not differ greatly, fermentation intensity and duration of " active fermentation " period showed less uniformity. In every case the higher peak point occurred in the T ciders, but fermentation ended sooner. The onset of fermentation was not uniform, however, for the 60° C. treatment ; the T sample began to ferment sooner, while the reverse happened with the 80° C. sample : for the 70° C. ciders appearance of fermentation was simultaneous.

Under treatment G the respective graphs show a very fair degree of uniformity, although not quite as close as those for treatments C and H. This similarity is very marked and the only difference is the extra day of fermentation vigour shown by graph H.

Discussion and Summary.

The work described in this paper has demonstrated that the Mo content of apple juice can be increased by injection of sodium molybdate into the branches of apple trees. The crops on the injected branches have shown an increase in Mo in the year of injection and the levels increased in fruit harvested the following year. The fruit on the untreated branches of the injected tree showed no increase in Mo content in the first year, but an increase in the second year.

Apart from the Mo changes, the analyses of the juices showed no significant differences from those of the untreated trees. The variations in flavour, specific gravity and rate of fermentation were no greater than those liable to occur with any series of untreated trees. The highest rate of

fermentation was from fruit borne on the Mo treated branches. The Mo content of the juices does not appear to have any direct bearing on the natural incidence of cider sickness.

The *freshly expressed* juices when subjected to a series of heat treatment tests followed by inoculation with the sickness bacillus have given responses in behaviour according to the nature of the treatment. With only one exception their response to infection with the bacillus has been very similar, irrespective of Mo content of the juices. On the other hand, the same juices subjected to similar tests *after they had undergone primary natural yeast fermentation* showed marked differences between those of high and low Mo content. These results were confirmed in other tests which indicated that a high Mo content is associated with changes in the nutrient complex of the ciders during or after the course of normal yeast fermentation, rendering the liquor more unfavourable for the growth of the sickness organism.

The possibility of the Mo content being toxic and therefore directly responsible for the results is excluded by the fact that the organisms have grown freely in a synthetic nutrient medium containing as much as 100 p.p.m. of that element.

It is shown that the Mo contents of the juices, as determined by the *Aspergillus* technique, change after sterilisation. The Mo content of the 1949 White Jersey and Yarlington Mill juices differed but slightly before and after sterilisation, when analysed very shortly after the expression of the juice. The available Mo in the sterilised White Jersey juice, however, when again analysed after standing for three months under sterile conditions, showed a marked decrease. With the 1950 Yarlington Mill juices sterilisation of the freshly expressed samples from the treated tree resulted in a material increase in the available Mo. Three months later, however, the sterilised juice from the treated branches contained only half the Mo content of the fresh juice. Such reduction of soluble Mo suggests a gradual precipitation by the formation of an insoluble compound, or compounds, with other juice constituents belonging to the microbiological nutrient complex, thus rendering the liquor less favourable for the growth of the sickness organism. This may explain the more drastic influence of the high Mo.

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THE EFFECT OF FRUIT STORAGE ON APPLE JUICE PROCESSING. II.

By MARGARET E. KIESER and A. POLLARD.

The possible application of fruit storage as a means of reducing the acidity of culinary apple varieties intended for juice processing was discussed in the last Report (1). Against the advantage of a lower acidity in the juice are to be set the disadvantages of fruit wastage in store, a loss of sugar, and possible deteriorations in flavour and stability of the juice. Tests carried out in the 1949–50 season suggested that a high acid variety of good keeping quality such as Bramley's Seedling could be stored until the acidity of the juice had fallen appreciably without undue deterioration in flavour and without an increased formation of deposits in the bottled juice. On the other hand, storage of the two dessert varieties resulted in an increased liability to the formation of pectin deposits in bottle. The tests have been continued in the 1950–51 season.

Experimental.

The varieties chosen for test were Bramley's Seedling, Lane's Prince Albert, Edward VII and Newton Wonder, with the dessert variety Laxton's Superb taken for comparison. Samples for pressing were taken from a store at 3–5° C. at intervals of approximately six weeks during the period from October to April. The juices were prepared as in the previous tests with the exception that clarification was effected by the addition of pectin-decomposing enzyme followed after two hours by a gelatin-tannin treatment : in the previous season gelatine fining had been omitted. The enzyme treatment used (0·1% Pectozyme for 20 hours at about 2° C.) was the same for all juices irrespective of the amount of pectin present in the fresh juice. The additions of tannin (tannic acid) and of gelatine were, however, adjusted to give maximum fining in each individual juice. All juices were filtered, saturated with carbon dioxide, bottled under carbon dioxide and pasteurised in bottle as before.

Samples of the freshly pressed and of the bottled juices were taken for analysis and these analyses, with observations after periods of storage, are given in Table I.

The effect of fruit storage on juice composition.

The juice analyses show some variations which may be attributed to variability in the fruit samples, but the general effects of fruit storage are common to all varieties and may be summarised as follows :—

Specific Gravity. All juices showed a fall in specific gravity following storage of the fruit, the fall over the longest period being about seven degrees of gravity.

Sugars. While the proportion of reducing sugar to sucrose increased, the total amount of sugar present decreased with storage, the figures being in general agreement with those for specific gravity.

TABLE I

COMPOSITION OF APPLE JUICES AFTER DIFFERENT PERIODS OF FRUIT STORAGE.

Variety and stage of maturity		S.G.	pH	Acid % (as malic)	"Tannin" %	Sugar %		Pectin % (as calcium pectate)		Deposit formation in stored juice	
						Reducing	Total	Original Juice	Bottled Juice	20/8/51	7/1/52
Lane's Prince Albert	I. 19/10/50	1·042	3·00	1·19	0·090	6·66	9·07	0·0183	Trace	1	3
	II. 11/12/50	1·0415	3·02	1·00	0·108	6·99	8·70	0·065	0·043	0	1
	III. 22/ 1/51	1·0395	3·18	0·93	0·095	7·07	8·32	0·088	0·062	0	2
	IV. 7/ 3/51	1·0355	3·28	0·70	0·085	6·86	7·65	0·095	0·073	0	4
	V. 16/ 4/51	1·035	3·22	0·79	0·116	6·92	7·57	0·073	0·068	1	5
Bramley's Seedling	I. 26/10/50	1·045	3·06	0·99	0·083	6·96	9·67	0·059	0·030	1	3
	II. 18/12/50	1·043	3·08	1·06	0·097	6·82	8·88	0·088	0·058	1	2
	III. 24/ 1/51	1·037	3·19	0·77	0·076	6·68	7·99	0·096	0·074	0	5
	IV. 12/ 3/51	1·039	3·21	0·82	0·097	6·99	8·10	0·107	0·083	1	2
	V. 16/ 4/51	1·039	3·25	0·62	0·095	7·20	8·01	0·110	0·079	1	5
Edward VII	I. 26/10/50	1·046	3·08	1·01	0·071	6·62	8·89	0·009	Trace	1	2
	II. 18/12/50	1·0415	3·22	0·84	0·074	6·96	9·07	0·120	0·084	0	2
	III. 24/ 1/51	1·0405	3·24	0·76	0·080	7·03	8·81	0·140	0·113	0	<1
	IV. 12/ 3/51	1·039	3·36	0·66	0·077	7·18	8·26	0·150	0·113	0	<1
	V. 16/ 4/51	1·040	3·41	0·61	0·082	7·49	8·64	0·180	0·129	0	1
Newton Wonder	I. 19/10/50	1·039	3·22	0·68	0·055	6·94	9·07	0·008	Trace	1	1
	II. 11/12/50	1·040	3·28	0·52	0·075	7·87	9·20	0·046	0·023	0	1
	III. 22/ 1/51	1·039	3·44	0·54	0·060	7·78	8·75	0·068	Trace	0	2
	IV. 7/ 3/51	1·038	3·57	0·41	0·060	7·93	8·71	0·064	0·038	0	3
	V. —	NO STORAGE									
Laxton's Superb	I. 19/10/50	1·048	3·42	0·66	0·048	7·51	10·49	0·154	0·083	1	4
	II. 11/12/50	1·044	3·54	0·48	0·057	7·89	9·63	0·166	0·120	1	1 mm. ppt.
	III. 22/ 1/51	1·0465	3·64	0·49	0·060	8·14	9·87	0·227	0·172	0	0·5 ,, ,,
	IV. 7/ 3/51	0·0455	3·86	0·36	0·051	8·52	9·67	0·234	0·179	2	6 ,, ,,
	V. —	NO STORAGE									

(1) deposit just perceptible.
(2) slight deposit.
(3) definite deposit.
(4) heavy deposit.
(5) very heavy deposit.

Titratable Acidity. The titratable acidity showed a considerable change over the storage period, both for high- and low-acid varieties, the decrease being between 35 and 45%. The pH figures show a steady rise over the period.

Tannin ($KMnO_4$-reducing substances). This figure showed comparatively little change over the period.

Pectin. In all varieties the amount of pectin passing into the juice increased with the length of fruit storage and in the culinary varieties the greatest increase occurred between the first and second samplings. The Laxton's Superb juice had a high pectin content at the first pressing in October and this increased still further after longer storage of fruit.

Flavour Tests. The juices as a whole had rather low specific gravities and their original flavours were poor and, with the exception of Laxton's Superb, too acid for palatability. Storage of the fruit gave a better sugar-acid balance where the acidity was originally high, but gave excessively sweet juices in Newton Wonder and Laxton's Superb. Storage of Bramley's Seedling and Lane's Prince Albert gave no marked deterioration in flavour until the last pressing in April : in the other varieties a stale character was evident in the juices pressed after December.

The effect of fruit storage on juice processing.

Clarification. The amounts of pectin left in the main bulks of juice after the standard clarification procedure, and still capable of being estimated as calcium pectate, corresponded with the amounts of pectin originally present in the fresh juice. The pectin contents of the bottled juices therefore increased with longer periods of fruit storage. Other tests on the freshly pressed juices, using graded amounts of enzyme showed, however, that the amounts of enzyme sufficient for clarification or viscosity reduction were not consistently related to the pectin content of the juices.

Storage deposits in bottle. The bottled juices were observed after periods of storage in a cool cellar and the extent of sedimentation found is indicated in the Table. In the summer following pressing (August, 1951) appreciable deposits were only seen in the later Laxton juices. The juices of the other varieties were free from haze and only very faint deposits were visible in individual batches. In the following January (1952) deposits were more apparent and varied from the just perceptible (Score 1) to a heavy deposit (Scores 4–5) which could be dispersed as a cloud in the body of the liquid.

Since the batches of fruit had been milled over a period of some months, the effect of fruit storage on juice stability is best seen by a comparison of samples which had been in bottle for similar lengths of time. In August, after they had been in bottle for 8 to 10 months, the juices of Lane's Prince Albert from the first and second pressings showed little or no sedimentation. The juices from the last two pressings, however, after a similar period of storage (January) showed very definite deposits in bottle. In Bramley's Seedling, deposit formation was greatest in juices III and V which showed darkening : in juice IV the amount was small and little greater than in juices I and II after a similar period of storage. The Edward VII juices showed no appreciable sedimentation after 8 to 10 months' storage in bottle. The juices from the four pressings of Newton Wonder showed increased deposition with increasing length of fruit storage, the last two juices showing both sedimentation and darkening.

In contrast to the foregoing juices, the later ones from Laxton's Superb gave voluminous white deposits and the juice from the last pressing had a heavy white precipitate about six millimetres in depth. The first two juices showed little deposition after 8 to 10 months in bottle, but on longer storage deposition became considerable.

Discussion.

The main factors which have been held responsible for the appearance of deposits in bottled apple juices are the precipitation of degraded pectin derivatives and the formation of insoluble oxidation or condensation products derived from juice tannins and related compounds. In the present series of juices, which were bottled immediately after clarification, many still contained appreciable amounts of pectinous material, and the deposition of pectin sediments on storage would therefore be expected. Such deposits were encountered in the juices of Laxton's Superb, especially from the later pressings. A sample of the heavy deposit in the juice from the last pressing was collected for analysis and amounted to 0·2 g. per half-pint bottle. It consisted almost entirely of partially de-esterified pectin, the calcium pectate figure being equivalent to 96·3% of the dry weight and the methoxyl value being 3·4% of the calcium pectate figure. The precipitate thus resembled that found in the Cox's Orange juice in the previous season (1).

The deposition in the Laxton juice in this season and in the dessert varieties in the previous season may be attributed to a high pectin content combined with residual pectin esterase activity. These varieties have been found to show a high enzyme activity and other work ((2) and unpublished data) has shown that some activity may remain after the normal bottle pasteurisation, especially in juices with pH values above 3·5. As the conditions favouring pectin deposit formation are present in juices derived from dessert varieties of this type, further pectin degradation, leading to sediment formation is therefore likely in such bottled and pasteurised juices if pectin has not been completely removed.

Heavy deposits of this nature were not found in the juices of the culinary varieties tested and the amount of sedimentation which did occur was not consistently related to the amount of pectin left in a juice at the time of bottling. The juices from Edward VII, which showed the least deposition on storage, had at the later pressings the highest pectin contents in the culinary fruit series. In this variety, the behaviour of the juices in bottle was little affected by the length of storage of the fruit before pressing. Prolonged storage of Lane's Prince Albert and Newton Wonder increased the tendency to deposit formation in the bottled juices. In Bramley's Seedling the effect of fruit storage was less definite.

The precipitates thrown down in juices of these culinary varieties were variable in type, being mainly of pale flocculent material, but in individual batches the juice showed darkening and the deposit appeared to resemble more closely the oxidation deposits described by Marshall (3). The extent of darkening may have been influenced by variations in the degree of carbonation of the samples, as found in other tests (1), but there was a tendency for this darkening to appear mainly in the juices pressed later than January.

In Continental practice it has been generally recommended that fruit for juice production should be harvested when ripe and processed without

delay (4), although some storage of the more acid varieties may, on occasion, be permissible (5). The condition suggested by Mehlitz (6), that fruit for juice production should be eatable in the raw state is, however, not met by English culinary varieties as normally harvested. It would probably be preferable to use for juice only those varieties which would individually give a juice already fairly balanced in acid and sugar content. The blending of high- and low-acid apple varieties may in itself increase processing difficulties, for such varieties may differ widely in their composition and in their physiological behaviour and their optimum states of maturity for processing may be reached at widely different times.

The results of the present tests make it evident that the use of some dessert varieties in juice blends requires that particular attention be paid to the removal or stabilisation of pectin in the juices and that pressing of the dessert varieties should not be delayed after harvest. Although instability in bottle may be lessened by a period of bulk juice storage, the increased pectin content of juices from stored dessert fruit is a major disadvantage during processing. The behaviour of the juices prepared from culinary fruit suggests, on the other hand, that the time of pressing is less critical for these varieties and that long-keeping varieties such as Edward VII may be stored until the original acidity has fallen some 30% without marked deleterious effects on juice stability. From the point of view of general juice quality, however, a shorter period of storage giving a more moderate fall in acidity would probably be advisable.

The tests of the two seasons therefore indicate that a restricted period of fruit storage offers a method for reducing the excessive acidity of high-acid culinary varieties as they are normally harvested.

Acknowledgments.

We wish to express our thanks to Miss M. E. Moseley and to Mr. P. E. W. Rogers for assistance in the experimental work.

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A COMPARISON OF SOME VARIETIES OF FRUIT PRESERVED BY BOTTLING, CANNING AND FREEZING.
PROGRESS REPORT II.

By ALICE CRANG and MARGARET STURDY.

A comparison of the qualities of different varieties of fruit preserved by bottling, canning and freezing has been continued during the 1951 season. The results are given for some varieties of soft fruit and for cherries.

Unless otherwise stated, the fruit was grown at Long Ashton Research Station.

Preserving Methods.

The bottling, canning and freezing methods were generally similar to those used in 1950 (1) except that in the 1951 season the bottled blackcurrants and gooseberries were processed by raising the water bath temperature to 180° F. in 1½ hours and maintaining it at that temperature for 15 min.

The fruit was preserved in sugar syrup, the proportions used being given under each kind of fruit.

Judging Methods.

The fruit was judged after five months' storage. The frozen fruit of kinds which are usually eaten raw was allowed to thaw for four hours at room temperature. The packets were then opened and the fruit judged against freshly opened bottles and cans. The frozen blackcurrants and gooseberries were cooked gently until tender in the syrup in which they were frozen, then cooled before tasting. The loganberries were judged both raw and cooked.

At least eight of a panel of nine people judged each of the fruits, which were put in identical glass bowls and marked by a code number for judging. The system of marking used was the same as in the previous year, a maximum of 10 marks allowed each for colour, texture and flavour, and 5 marks each for the sugar/acid balance and size of the fruit.

Results.

Blackcurrants.

The varieties were the same as those preserved in 1950, except that the seedling variety PS/24/1922 has been omitted from this season's results as it was under-ripe when preserved. The other varieties were in good condition, and left in a cool store overnight before preserving. The quantities used were 250 g. of fruit and 200 ml. of 40° Brix syrup in each container. The average total scores are given in Table I.

Processes.

The canned blackcurrants were significantly better than either the bottled or frozen fruit due largely to better texture and flavour. There was little difference in the marking for colour, and the sugar/acid balance was considered satisfactory for all these processes. The flavour of the frozen fruit was poorer than the bottled or canned fruit for each variety.

TABLE I.
BLACKCURRANTS.

Variety	Bottled (max. 40)	Canned (max. 40)	Frozen (max. 40)	Variety. Mean of all processes (max. 40)
Baldwin	29·8	31·1	28·5	29·8
Cotswold Cross ..	31·4	32·9	30·6	31·6
Malvern Cross ..	31·8	31·9	29·4	31·0
Mendip Cross ..	27·5	32·6	31·0	30·4
Seabrook	30·1	32·4	32·5	31·7
Westwick Choice ..	31·6	32·0	29·8	31·1
Process. Mean of all varieties	30·4	32·2	30·1	30·9

	$P < \cdot05$	$P < \cdot01$
Significant difference for mean of processes ..	0·63	0·85
,, ,, ,, ,, ,, varieties ..	0·99	1·35
,, ,, ,, varieties in one process	1·98	2·51

Processes : Canned > Bottled or Frozen ($P < \cdot01$).
Varieties : Seabrook, Cotswold Cross > Baldwin ($P < \cdot01$), Mendip Cross ($P < \cdot05$).
Bottled Fruit : Malvern Cross, Westwick Choice, Cotswold Cross > Mendip Cross ($P < \cdot01$).
Malvern Cross > Baldwin ($P < \cdot05$).
Canned Fruit : No significant difference between varieties.
Frozen Fruit : Seabrook > Westwick Choice, Malvern Cross, Baldwin ($P < \cdot01$).
Mendip Cross > Baldwin ($P < \cdot05$).

Varieties.

The differences in the varieties of blackcurrants tested were not great, but the following points were noted :—

Baldwin. This variety was slightly below the average for each method of preserving due to poorer flavour, though last year it was above the average in this respect.

Cotswold Cross. This fruit was again judged the best of the series for colour.

Malvern Cross. Quite a good variety, but not of outstanding quality.

Mendip Cross. The canned and frozen fruit were quite good, but the bottled fruit was disappointing in colour.

Seabrook. A good variety for each method of preserving.

Westwick Choice. Another good variety for heat-processing, not quite so good when frozen.

Cherries.

The cherries were obtained from Ledbury and were in good condition when preserved. The cherries were stemmed but not pitted for preserving. 250 g. of fruit and 200 ml. of 30° Brix syrup was used in each container. The average total scores are given in Table II.

TABLE II.

CHERRIES.

Variety	Bottled (max. 40)	Canned (max. 40)	Frozen (max. 40)	Variety. Mean of all processes (max. 40)
Black Tartarian ..	29·9	30·6	32·4	31·0
Frogmore Early ..	31·0	29·8	31·8	30·9
Governor Wood ..	28·7	29·4	33·8	30·6
Noir de Guben ..	28·8	30·9	32·0	30·6
Roundel Heart ..	29·8	30·2	31·9	30·6
Waterloo	28·2	30·9	30·1	29·7
Process. Mean of all varieties	29·4	30·3	32·0	30·6

	$P < ·05$	$P < ·01$
Significant difference for mean of processes ..	0·75	1·02
,, ,, ,, ,, ,, varieties ..	0·35	0·47
,, ,, ,, varieties in one process	2·10	3·02

Processes : Frozen > Canned and Bottled ($P < ·01$).

Varieties : All other varieties > Waterloo ($P < ·01$).

Bottled Fruit : Frogmore Early > Waterloo or Noir de Guben ($P < ·05$).

Canned Fruit : No significant difference between varieties.

Frozen Fruit : Governor Wood > Waterloo ($P < ·01$).

Processes.

The colour of the frozen fruit was the outstanding feature, being excellent when the fruit was just thawed, though the light variety soon developed brown discolouration on standing. In comparison the colour of the bottled fruit was poor and the canned fruit was very poor. It was on colour chiefly that the frozen fruit was judged significantly better than the bottled or canned fruit.

The texture of the bottled fruit was tougher than that of the canned or frozen fruit. The flavour was not considered to be very good in any of the processes. The proportion of fruit to sugar used was satisfactory.

Varieties.

Black Tartarian. The appearance of the frozen fruit was good, though opinions of the judges varied on the flavour. The canned fruit was poor in colour but quite good in other qualities.

Frogmore Early. This cherry was most attractive when first thawed, but became dull on standing and was fair in the heat-processed fruit. The flavour was slightly better retained in the canned and bottled than in the frozen fruit.

Governor Wood. This variety was judged the best of the series when frozen, being of a very good dark red colour, but in heat-processing, this became dull and unattractive. The flavour was poor in the bottled fruit, and below the average in the canned fruit.

Noir de Guben. The colour of this variety was very good in the frozen fruit, and though not good, it was the best of the series for the bottled and canned fruit. The texture was a little tough in each method of processing. The flavour was fairly good.

Roundel Heart. The colour was not so well retained in the frozen fruit as in the former varieties, but the other qualities were quite good.

Waterloo. For colour this variety was judged the poorest of the series in each method of processing. The flavour of the canned fruit was judged the best of the series. In other qualities it was intermediate.

Gooseberries.

The three varieties of gooseberries tested were those compared in previous years. The fruit was preserved while the berries were green and hard. The quantities used were 250 g. of fruit and 200 ml. of 30° Brix syrup in each container. The average judging scores are tabulated below.

TABLE III.

GOOSEBERRIES.

Variety	Bottled (max. 40)	Canned (max. 40)	Frozen (max. 40)	Variety. Mean of all processes (max. 40)
Keepsake	32·4	30·5	27·9	30·3
Leveller	33·4	33·5	29·5	32·1
Whinham's Industry	28·8	30·3	28·3	29·1
Process. Mean of all varieties	31·5	31·4	28·6	30·5

	$P < \cdot 05$	$P < \cdot 01$
Significant difference for mean of varieties or processes	1·12	1·50
„ „ „ varieties in one process ..	1·94	2·61

Processes : Bottled or Canned > Frozen ($P < \cdot 01$).
Varieties : Leveller > Keepsake ($P < \cdot 01$) > Whinham's Industry ($P < \cdot 05$).
Bottled Fruit : Leveller and Keepsake > Whinham's Industry ($P < \cdot 01$).
Canned Fruit : Leveller > Keepsake and Whinham's Industry ($P < \cdot 01$).
Frozen Fruit : No significant difference in varieties.

Processes.

The colour of the frozen fruit in each variety was judged slightly better than the bottled or canned fruit, but this was outweighed by the texture and flavour, in which the bottled and canned fruit was judged to be superior to the cooked frozen fruit. Although the same proportions of fruit and sugar were used for each process, the sugar had not penetrated so well in the frozen fruit, and this was judged to be too acid, whereas the balance of sugar and acid in the heat-processed fruit was considered to be satisfactory. These factors together caused the frozen fruit to be judged significantly poorer than the heat-processed fruit. There was no significance between the bottled and canned fruit. The higher processing temperature used this season for the bottled fruit had improved the texture.

Varieties.

Keepsake. This variety was pale in colour but otherwise satisfactory for bottling and canning. The colour and flavour of the frozen fruit were judged to be poorer than in the other varieties.

Leveller. This fruit gained the highest marks in each method of preserving.

Whinham's Industry. This has proved less satisfactory than the previous varieties for bottling, due to poorer texture and flavour. Differences in the canned and frozen fruit were not so great.

Loganberries.

The loganberries were preserved by bottling and canning and by freezing both in sugar syrup and in sugar, using 270 g. of fruit and either 190 ml. of 40° Brix syrup or its equivalent in sugar. The fruit and dry sugar was mixed in a bowl before freezing. The fruit frozen with sugar and one lot of the loganberries frozen with syrup were judged uncooked and the other loganberries in syrup were cooked and cooled before judging. All methods were judged together. The average scores of eight tasters are given in Table IV. The maximum marks for each is 40.

TABLE IV.

LOGANBERRIES.

Bottled	Canned	Frozen with Syrup (uncooked)	Frozen with Syrup (cooked)	Frozen with Sugar (uncooked)
33·0	32·4	29·9	29·8	29·1

	$P < \cdot 05$	$P < \cdot 01$
Significant difference	1·97	2·66

Bottled > all freezing methods ($P < \cdot 01$).
Canned > freezing with sugar ($P < \cdot 01$), freezing with syrup ($P < \cdot 05$).

It will be seen that the bottled and canned fruit was preferred to any of the frozen fruit. The colour and the size of the bottled and canned fruit was more even, and the texture slightly better than in the frozen fruit. The flavour was quite well retained in each method of processing, but the sugar had not penetrated so well in the frozen fruit and this was considered too acid.

A can and bottle of loganberries stored from the previous season had deteriorated considerably in colour and slightly in flavour when compared with the new season's fruit.

The cooked frozen fruit was marked higher in colour but lower in flavour than the uncooked fruit.

Strawberries.

Four varieties of strawberries were judged this season. The Axbridge Early strawberries were grown at Cheddar, the Huxley at Brockley and the Royal Sovereign sample in Long Ashton, but not at the Research Station. The Auchincruive Climax, Axbridge Early and Huxley varieties were in very good condition, but the Royal Sovereign berries were only of average quality.

The proportions used were 256 g. of fruit to 180 ml. of 40° Brix syrup. Artificial colouring ($\frac{1}{8}$% Ponceau 2R) was used in preparing the syrup for the bottled and canned fruit. The average tasting results are given below.

TABLE V.

STRAWBERRIES.

Variety	Bottled (max. 40)	Canned (max. 40)	Frozen (max. 40)	Variety. Mean of all processes (max. 40)
Auchincruive Climax ..	27·4	28·1	32·2	29·2
Axbridge Early ..	27·0	27·3	29·5	27·9
Huxley	30·1	29·4	31·2	30·2
Royal Sovereign ..	27·4	29·1	29·4	28·6
Process. Mean of all varieties	28·0	28·5	30·6	29·0

	$P < \cdot05$	$P < \cdot01$
Significant difference for mean of processes ..	0·85	1·12
,, ,, ,, ,, ,, varieties ..	1·70	2·24

Processes : Frozen > Bottled and Canned ($P < \cdot01$).
Varieties : Huxley > Axbridge Early ($P < \cdot05$).

Processes.

The colour, size and general appearance of the frozen fruit when first thawed was greatly superior to the heat-processed fruit. The flavour was also better in certain varieties. These factors together accounted for the frozen fruit being judged significantly better than the bottled or canned fruit. The colour of the bottled fruit was better than the canned fruit, but in some varieties both were too highly coloured, and the proportion of dye used could be reduced with advantage.

The flavour of the frozen fruit was preferred to the bottled or canned fruit of the same variety.

The proportion of sugar to acid was satisfactory in each process. Apart from colour there was little difference between the bottled and canned fruit.

Varieties.

Auchincruive Climax. This variety again was the best of the series for freezing, but the flavour though good in this process, was not considered to be so well retained in heat-processing as in the other varieties.

Axbridge Early. The colour and flavour were not very well preserved in this variety in comparison with the others.

Huxley. This variety gave the best results in the heat-processed fruits this year due to colour and flavour. The tendency to toughness noted in the bottled and canned fruit was very apparent in the frozen samples.

Royal Sovereign. The results were disappointing this season in the bottled and canned fruit, and once again it gave a poor appearance after freezing.

Discussion.

A comparison of the heat-processing and freezing qualities of some varieties of blackcurrants, gooseberries, loganberries and strawberries during another season has generally confirmed the findings given for the 1950 season (1) and by other workers in previous seasons (2) (3), but shows how essential it is to test the fruit during several seasons before any reliance is placed on the individual results. Six varieties of cherries were compared for the first time.

It was again found that freezing has no advantages over heat-processing methods after a short period of storage for fruit which needs cooking before use, but was superior for the dessert fruits tested and for cherries. Some fruit stored from the previous season indicated that the deterioration in the bottled and canned fruit over longer periods of storage was greater than in the frozen fruit.

Freezing would seem to be the only satisfactory method of preserving black cherries, which lose much of their colour in bottling or canning (2).

Summary.

The results of tasting tests on 20 varieties of soft fruits and cherries preserved by bottling, canning and deep freezing are given.

The frozen fruit was again found to be generally superior to the heat-processed fruit in colour, but frequently inferior in texture or flavour.

Acknowledgments.

Our thanks are due to Miss H. Ferres for assistance in the statistical treatment of the results, to Miss Hooper and Miss Hart for assistance in the experimental work, and to all the members of the tasting panel.

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A COMPARISON OF SOME VARIETIES OF VEGETABLES PRESERVED BY BOTTLING, CANNING AND FREEZING. PROGRESS REPORT I.

By ALICE CRANG and MARGARET STURDY.

A comparison has been made of the colour, flavour and texture of some varieties of vegetables after preservation by bottling, canning or freezing under domestic conditions during the 1950 and 1951 seasons.

Experimental.

Vegetables Used.

Most of the varieties were grown at Long Ashton Research Station and preserved at the stage of optimum eating quality. A note is made of vegetables

obtained from other sources. Every endeavour was made to preserve the vegetables as soon as they were picked.

Heat-processing Methods.

The bottled and canned vegetables were preserved either in 1 lb. glass preserving jars or in A2½ vegetable lacquered cans. Both were processed at 10 lb. steam pressure for the requisite time,* and stored in semi-darkness at room temperature.

Freezing Methods.

The prepared vegetables were scalded in boiling water, using sufficient water so that it would re-boil within a minute of adding the vegetable, and boiled for the time recommended. They were then drained and cooled to room temperature in ice cold water. The drained vegetables were either frozen alone or with cold 2% brine in packets made from "Cellophane" grade MSAT 300/30. The usual precautions were taken to squeeze out as much air as possible before heat-sealing the packets, which were then placed in waxed cardboard cartons for freezing. The temperature of the cabinet used for the freezing and storage was between –10° and 0° F.

Judging Methods.

The frozen vegetables were taken from their packets and cooked at once either in the brine in which they were frozen or in an equal quantity of boiling 2% brine. When the vegetables were partly thawed, the packs were broken up to aid even heating. The additional cooking time required was found to be about half that required for the fresh vegetable.

The bottled or canned vegetables were taken from the containers and re-heated just prior to judging. All the vegetables were put into identical containers and judged in the same way as fruit (1) (2), except that only colour, texture and flavour were marked, giving a maximum of 30 marks obtainable. A separate note was made of any vegetables considered either too salt or not salt enough.

Results.

Asparagus.

The asparagus was grown at Evesham and preserved the day after picking. The prepared stems were scalded for 3 min. before preserving, and the frozen vegetable cooked for a further 5 min. in boiling water before serving. A comparison was made between fresh asparagus and the bottled and frozen samples stored for 11 months. The averages of five markings are tabulated on opposite page.

The colour of the frozen asparagus was considered almost as good as the fresh, but that of the bottled was poor. The texture of the frozen product was poorer than the bottled, and distinctly poorer than the fresh asparagus. In flavour, the fresh was marked slightly above the average, the frozen in both samples slightly below average, and in comparison the bottled asparagus was considered very poor.

There was scarcely any difference between the asparagus frozen with or without brine.

* See Ministry of Agriculture Bulletin 21, 7th Edition, for processing details.

TABLE I.

ASPARAGUS : Marks given (out of 30).

Bottled	Frozen	Frozen in brine	Fresh
15·4	23·2	23·2	27·8

	$P < \cdot05$	$P < \cdot01$
Significant difference	1·92	2·68

Fresh > Frozen, either process ($P < \cdot01$) > Bottled ($P < \cdot01$).

Beans (Broad).

Triple White beans were preserved in the two seasons and Eclipse, Essex Wonder and Harlington (white) during the second season. The beans were podded and scalded for 3 min. in boiling water before heat-processing or freezing. They were compared after 4 months' storage. The mean of seven judging scores for 1951 is given in Table II.

TABLE II.

BEANS (BROAD) : Marks given (out of 30).

Variety	Bottled	Canned	Frozen	Variety. Mean of all processes
Eclipse	18·6	19·3	22·9	20·2
Essex Wonder ..	18·4	19·4	23·4	20·4
Triple White ..	22·7	22·4	25·0	23·4
Harlington ..	18·4	19·9	22·9	20·4
Process. Mean of all varieties	19·5	20·2	23·6	21·1

	$P < \cdot05$	$P < \cdot01$
Significant difference for mean of processes ..	0·94	1·27
,, ,, ,, ,, varieties ..	1·09	1·46
,, ,, ,, varieties in one process	1·88	2·53

Process : Frozen > Bottled or Canned ($P < \cdot01$).
Varieties : Triple White > all other varieties ($P < \cdot01$).
Bottled : ,, ,, ,, ,, ,,
Canned : ,, ,, ,, ,, ,,
Frozen : ,, ,, Eclipse and Harlington ($P < \cdot05$).

Processes.

There were no significant differences between the effects of bottling and canning. In colour the frozen beans were greatly superior to those bottled or canned in each variety, but with Eclipse, Essex Wonder and Harlington, the brown colour of the heat-processed vegetables was very unappetising. The flavour of the frozen beans was better than the bottled or canned ones in each variety, but there was little difference in texture, all products being considered satisfactory.

Varieties.

Eclipse, Essex Wonder, Harlington. These varieties were very similar in all their markings, being of average quality when frozen. The development of a brown colour on heat-processing renders them unsuitable for bottling or canning.

Triple White. This is a recommended variety for bottling or canning as the beans turn a pale green colour when heat-processed. It also proved the best of the varieties tested for freezing. The results of the previous year's preserving were very similar, but as it was not compared with other varieties, details of the marks were not given.

Beans (Dwarf and Runner).

Two varieties, Masterpiece and Princeps, were preserved during 1951 by bottling, canning and freezing. The Masterpiece beans were bottled and canned whole, and frozen both whole and in slices. The variety Princeps was preserved in slices for each process. The canned and bottled beans had 0·005% of pea green colour added to the brine. The beans were judged after 4 months' storage and the following are the mean of 8 scores for each process.

TABLE III.

Beans (Dwarf and Runner): Marks given (out of 30).

Variety	Bottled	Canned	Frozen
Masterpiece (Whole) ..	22·0	19·4	21·0
„ (Sliced) ..	—	—	23·8
Princeps „ ..	22·1	20·6	25·8

	$P < \cdot 05$	$P < \cdot 01$
Significant difference for Masterpiece ..	1·39	1·88
„ „ „ Princeps ..	3·26	4·42

Masterpiece, frozen sliced > frozen whole or canned ($P < \cdot 01$) and bottled ($P < \cdot 05$).

Princeps, frozen > canned ($P < \cdot 01$) bottled ($P < \cdot 05$).

Processes.

The most striking difference was noted in the colour; the sliced frozen beans both retained this very well, but the whole frozen beans were intermediate in colour between the canned and bottled beans, the canned beans of both varieties being the poorest. There was no great difference in texture resulting from the different processes.

The flavour of the frozen beans in each process was rated higher than the bottled or canned beans of the same variety.

Varieties.

In view of the difference in colour noted between the whole and sliced frozen beans in the variety Masterpiece, no direct comparison of the other processes can be made. Both varieties are suitable for freezing when sliced.

Mushrooms.

Cultivated " button " mushrooms in good condition were obtained from Wrington, Somerset. Some were processed unpeeled on the day of picking, and others picked and peeled the day before processing. Two methods of canning were used : in one the peeled mushrooms were canned in brine, and in the other the mushrooms were cooked in a casserole before canning.* The frozen mushrooms were scalded in boiling water, taking 4 minutes for the water to re-boil, and boiled a further 4 min., cooled in ice cold water and frozen either with or without brine. They were judged after 9 months' storage. The mean score of the 5 judges is given in Table IV.

TABLE IV.

Mushrooms : Marks given (out of 30).

Canned. Peeled in brine	Canned. Unpeeled. Pre-cooked in casserole	Frozen. Whole in brine	Frozen. Whole without brine
22·8	21·2	14·6	13·0

	$P < \cdot 05$	$P < \cdot 01$
Significant difference	3·82	5·21

Canned, both methods > Frozen, both methods ($P < \cdot 01$).

The outstanding difference in the frozen and canned methods was in texture, the frozen mushrooms being extremely tough even after prolonged stewing.

It is hoped to repeat tests on this vegetable using sliced instead of whole mushrooms, and to see if alternative methods of cooking give better results.

Peas.

During 1950 two varieties, Onward and Petit Pois, were tested, and in 1951 five varieties, Foremost, Lincoln and Thomas Laxton, in addition to those previously mentioned. All the varieties were preserved by bottling, canning and freezing. The bottled and canned peas had 0·005% pea green colour added to the brine used in preserving. No artificial colour was used for the frozen peas.

For freezing the peas were scalded for 2 min. in boiling water and in the first season frozen with and without brine. No advantage was found in freezing with brine, so this was omitted for the second season.

The mean scores given in Table V are those of 5 judges in 1950 and 8 in 1951, judged after the peas had been stored for 10 and 5 months respectively.

Processes.

In both seasons the frozen peas of each variety have been judged significantly superior to the bottled or canned peas, due chiefly to the much better colour of the frozen product, but also to the better texture and flavour. After comparison between the heat-processed and frozen peas, the differences between the bottled and canned peas were small, but there was a slight preference for the bottled over the canned peas as regards flavour.

* For details see Bulletin No. 21, 7th Edition.

TABLE V.

PEAS : Marks given (out of 30).

Variety.	Season	Bottled	Canned	Frozen	Variety. Mean of all processes
Onward	1950	16·8	18·0	27·6	20·8
Petit Pois ..	„	21·6	20·0	24·6	22·1
Process. Mean of both varieties	„	19·2	19·0	26·1	21·5
Foremost ..	1951	18·3	16·4	26·0	20·2
Lincoln	„	21·1	20·0	23·9	21·7
Onward	„	21·4	20·6	28·5	23·5
Petit Pois ..	„	19·8	18·0	25·0	20·9
Thomas Laxton..	„	21·1	21·3	27·5	23·3
Process. Mean of all varieties	„	20·3	19·3	26·2	21·9

	$P < \cdot05$	$P < \cdot01$
Significant difference for mean of Processes—1950	1·33	1·78
„ „ „ „ „ Varieties—1950	1·08	1·46
„ „ „ Varieties in one process—1950 ..	1·87	2·53
„ „ „ mean of Processes—1951	0·69	0·83
„ „ „ „ „ Varieties—1951	0·89	1·19
„ „ „ Varieties in one process—1951 ..	1·54	2·07

1950—Frozen > Bottled or Canned ($P < \cdot01$).
1951—Frozen > Bottled ($P < \cdot01$) > Canned ($P < \cdot01$).
1950—Bottled or Canned : Petit Pois > Onward ($P < \cdot05$).
1951— „ „ „ Lincoln, Onward, Thomas Laxton > Foremost ($P < \cdot01$).
1950—Freezing : Onward > Petit Pois ($P < \cdot01$).
1951— „ Onward > Foremost, Lincoln and Petit Pois ($P < \cdot01$).
„ „ Thomas Laxton > Lincoln, Petit Pois ($P < \cdot01$).

Varieties.

The conflicting results when comparing the varieties Onward and Petit Pois during the two seasons emphasise the danger of drawing conclusions on one season's scores. In the second season the variety Petit Pois was judged by the tasting panel to be over-mature.

Foremost. The flavour of this variety was judged to be the poorest of the series in each method of preserving. It was much more suitable for freezing than heat-processing.

Lincoln. This variety, which is recommended for canning, was, because of its lighter colour, the least satisfactory of the series when frozen.

Onward. This variety was excellent in colour when frozen and very good in flavour and texture. The colour of canned and bottled peas stored for 10 months was, however, very poor, and that of the peas stored 5 months only fair. The flavour and texture of the heat-processed peas were fairly good.

Petit Pois. Quite good when frozen, but the colour was not outstanding. In this quality, however, the bottled peas were better than other varieties. The flavour was quite good in the frozen peas, but poor in the bottled and canned ones. The texture was good in all processes, in spite of the peas appearing to be over-mature in the second season.

Thomas Laxton. In the one season's trials this variety was rated very good when frozen and quite good when bottled and canned, being considered the best of the series for flavour when canned.

Discussion.

Vegetable preservation by freezing has been judged in these trials to be greatly superior to bottling or canning for all the vegetables examined with the exception of mushrooms, in which the texture of the frozen vegetable proved very tough even after prolonged boiling. The great advantage of freezing green vegetables is that the natural bright green of the chlorophyll is retained. The unattractive colour produced by the high temperatures required for vegetable bottling and canning cannot be completely masked by the use of artificial colourings. The frozen vegetables were also found to be better in flavour and texture than the same variety when heat-processed, but the differences were not so marked.

From the view-point of the domestic preserver, freezing vegetables, wherever practicable, would seem more satisfactory than canning or bottling, both of which entail processing for a considerable time under carefully controlled steam pressure. As the vegetables have to be scalded before preserving in all the methods compared, there would be considerable saving in time in freezing over canning or bottling. The question of comparative cost under domestic conditions has not been considered so far.

Although the choice of the most suitable varieties of vegetables for freezing is a matter that should be considered, it may not be quite so important as in canning. Varieties recommended for canning are often not the best for freezing, as shown also by Campden Research Station (3).

The choice of vegetables at the optimum stage of growth and their quick preparation will undoubtedly be required to obtain satisfactory results in freezing as in heat-processing. The method of preparation of the mushrooms and dwarf beans needs further investigation. Freezing the vegetables in brine seems to hold no advantages over freezing them alone.

The temperatures of freezing and storing (–10° to 0° F.) seemed quite satisfactory for vegetables, and gave products that compared very favourably with those commercially quick-frozen.

It must be emphasised that in this investigation most of the varieties of vegetables have been compared for their preserving qualities during one season only. Even so, the advantages of frozen over heat-processed vegetables seem very marked in most of varieties tested, whereas there was comparatively little difference between the bottled and canned vegetables.

Summary.

A comparison has been made of the colour, texture and flavour of some varieties of vegetables preserved by deep freezing, bottling and canning under

domestic conditions. All the frozen green vegetables were judged to be greatly superior to the heat-processed ones. Different qualities are required for obtaining satisfactory frozen and canned vegetables.

Acknowledgments.

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METEOROLOGICAL RECORDS, 1951

Month	Air Temperature (°F.)							Rainfall (″)			Sunshine (hours)			Soil Temperatures at 09.00 G.M.T.		
	Means		Mean of A & B	Dif. from average	Max.	Min.	No. of ground frosts	Total	Dif. from average	Most in a day	Total	Dif. from normal	Daily average	4″	8″	24″
	A Max.	B Min.														
January	45·8	35·5	40·7	+0·1	45	23	16	4·27	+0·72	0·93	36·2	—11·7	1·17	39·0	39·4	41·5
February	45·2	34·9	40·1	—0·6	50	30	18	4·35	+ 1·81	0·77	70·4	+ 13·3	2·51	38·2	38·8	41·1
March	46·7	35·5	41·1	—2·8	55	28	13	4·13	+1·79	0·79	82·2	—40·4	2·65	39·9	40·0	42·3
April	53·5	37·4	45·4	—2·4	71	30	16	3·80	+1·55	0·79	189·9	+ 30·5	6·33	45·1	44·6	44·7
May	58·0	43·1	50·5	—2·5	67	34	6	2·94	+0·42	0·91	154·8	—40·5	4·94	50·5	47·9	50·7
June	67·4	49·0	58·2	—0·2	75	42	0	0·68	—1·46	0·18	252·8	+40·1	8·43	60·7	59·3	58·7
July	71·4	53·6	62·5	+0·8	80	43	0	1·66	—1·60	0·51	217·5	+21·4	7·02	65·5	64·4	62·5
August	66·2	51·8	59·0	—2·1	72	41	0	5·10	+1·69	1·13	170·2	—15·5	5·49	60·5	60·4	62·1
September	62·6	49·1	58·2	+0·9	74	41	0	3·02	—0·05	0·72	88·2	—53·2	2·94	58·3	57·7	58·5
October	58·1	42·9	50·5	—0·6	64	27	7	0·75	—3·13	0·33	111·9	+11·1	3·61	50·3	50·9	55·1
November	53·6	42·6	48·1	+3·5	59	29	5	7·24	+3·69	0·99	68·1	+11·0	2·27	46·3	46·9	50·1
December	49·7	37·7	43·7	+2·3	56	25	14	2·50	—1·29	0·48	61·7	+15·6	1·97	41·9	42·6	46·1
Total or Mean			49·8	—0·3			95	40·44	+4·14		1503·9	—28·3	4·12	49·7	49·4	51·1

G. E. Clothier.

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